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The American Journal of Medicine

Vol. XIII SEPTEMBER, 1952 No. 3

Editorial

Clinical Studies

In this elegant analysis of the cause of a congenital coagulation defect in three siblings the authors could demonstrate that the difficulty was not due to apparent hypoprothrombinemia but to a deficiency of AC-globulin, readily corrected by transfusion of fresh whole blood or plasma. The paper includes a lucid discussion of the distinct factors normally present in plasma (Ac-globulin) and serum ("SPCA") which are both required to accelerate conversion of prothrombin to thrombin. A revised classification of the true hypoprothrombinemias and pseudohypoprothrombinemias is offered.

This stimulating paper brings into better focus the dynamic relationship between life span of the red cell and the rate of erythropoiesis as determinants of the circulating red cell mass. If the limits of this equilibrium are exceeded under the stress of hemolytic disease, anemia results. Thus the authors, using the Ashby method and related technics, were able to show in two cases of hereditary hemolytic anemia that with reduction of average red cell survival to less than fifteen to twenty days, hemoglobin production increased about seven-fold and yet anemia developed. Other related observations add to the interest of the study.

There is some question whether vitamin B_{12} therapy of pernicious anemia is of itself wholly adequate treatment—whether some other antipernicious anemia principle in liver may also be required for optimal results in treatment. The present study, a careful evaluation of long-term therapy with vitamin B_{12} in a large number of cases of pernicious anemia, indicates that vitamin B_{12} alone is as effective as refined liver extract in producing and maintaining remission in the clinical and hemotologic manifestations of pernicious anemia, and is the preferred form of treatment.

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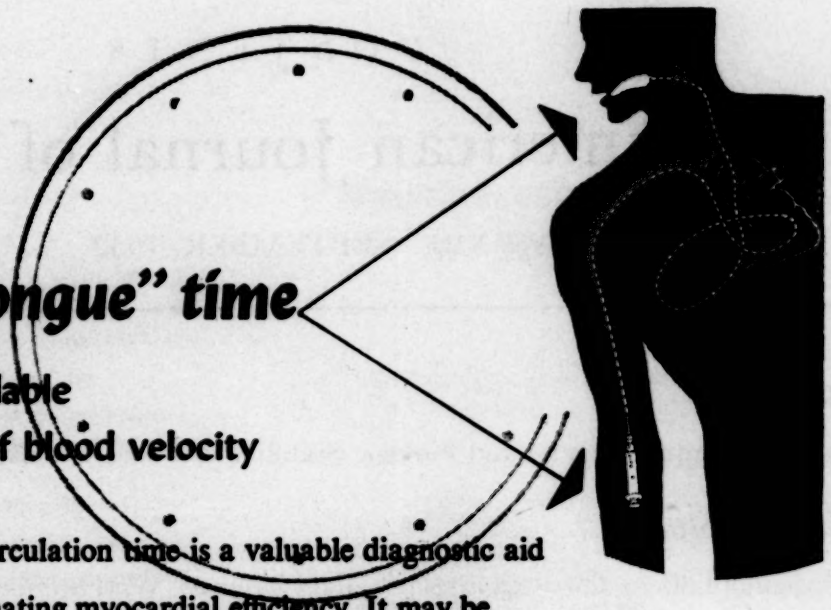

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Blood circulation time is a valuable diagnostic aid for estimating myocardial efficiency. It may be determined simply and dependably with *Decholin Sodium*. While the patient is lying quietly, inject 3 to 5 cc. of *Decholin Sodium* rapidly (2 to 3 seconds) through an 18-gauge needle into the cubital vein. The "arm-to-tongue" circulation interval is timed from the start of the injection to the patient's perception of a bitter taste. The average "arm-to-tongue" time is from 9 to 16 seconds.

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The American Journal of Medicine

Vol. XIII SEPTEMBER, 1952 No. 3

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new, fast-acting analgesic containing acetyl-p-aminophenol

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Trigesic with Codeine, *per tablet:*

16 mg. (¼ gr.) or 32 mg. (½ gr.) codeine phosphate in addition to the other ingredients. Bottles of 100 and 1,000 pink, scored tablets on prescription only.

TRIGESIC

Squibb Analgesic Compound

SQUIBB

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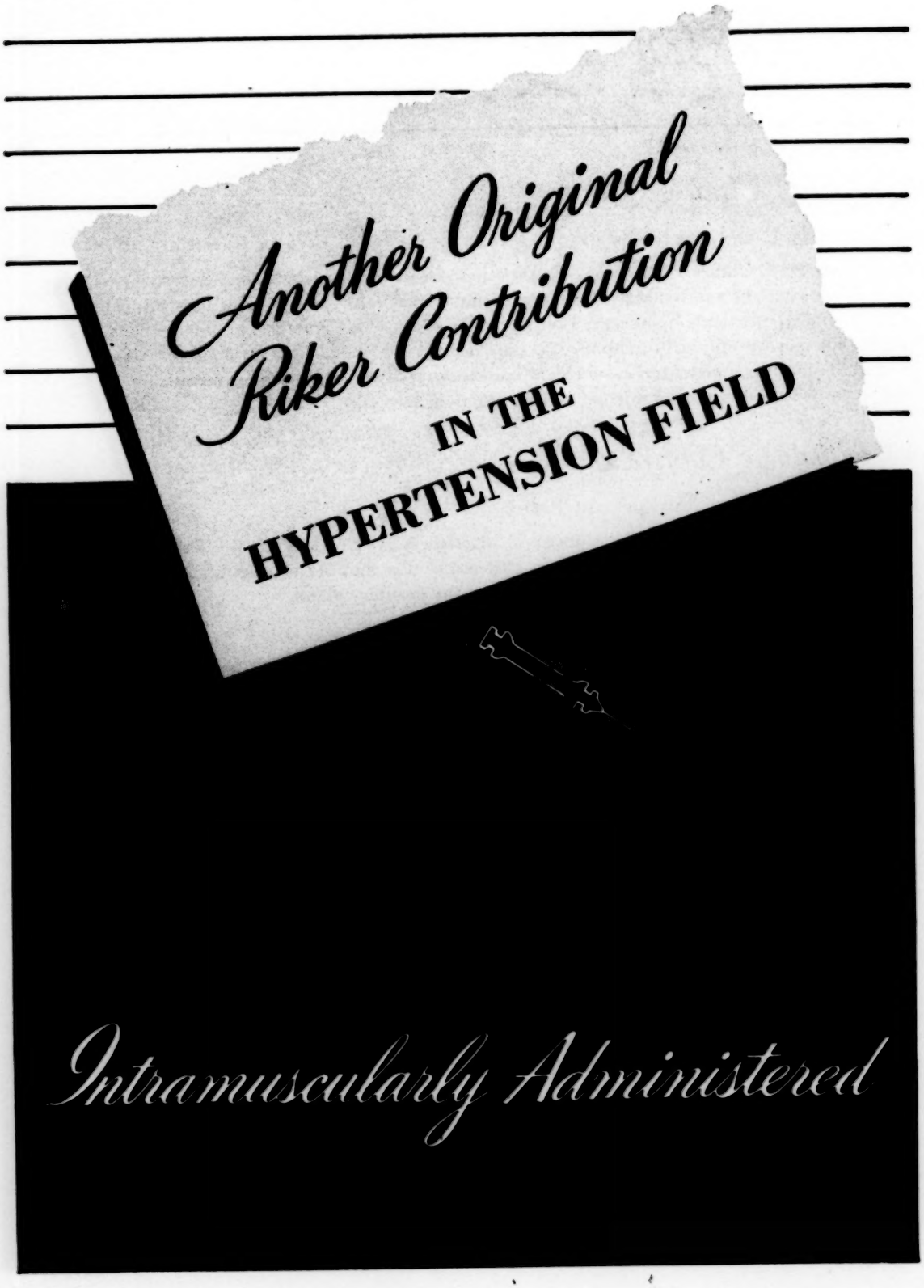
Change of address must reach us one month preceding month of issue.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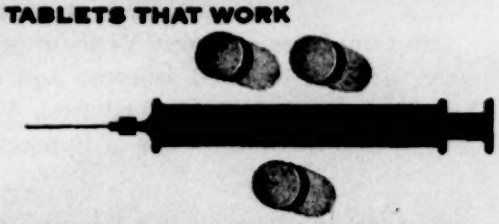

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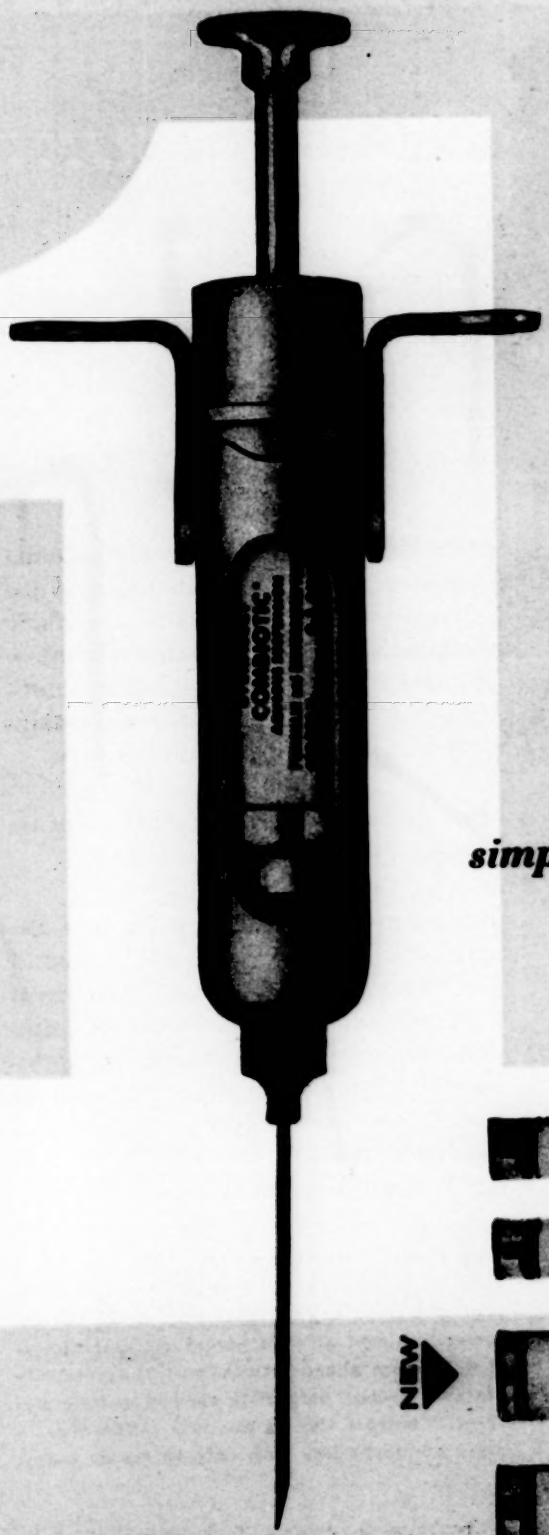

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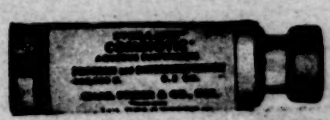

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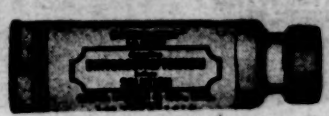

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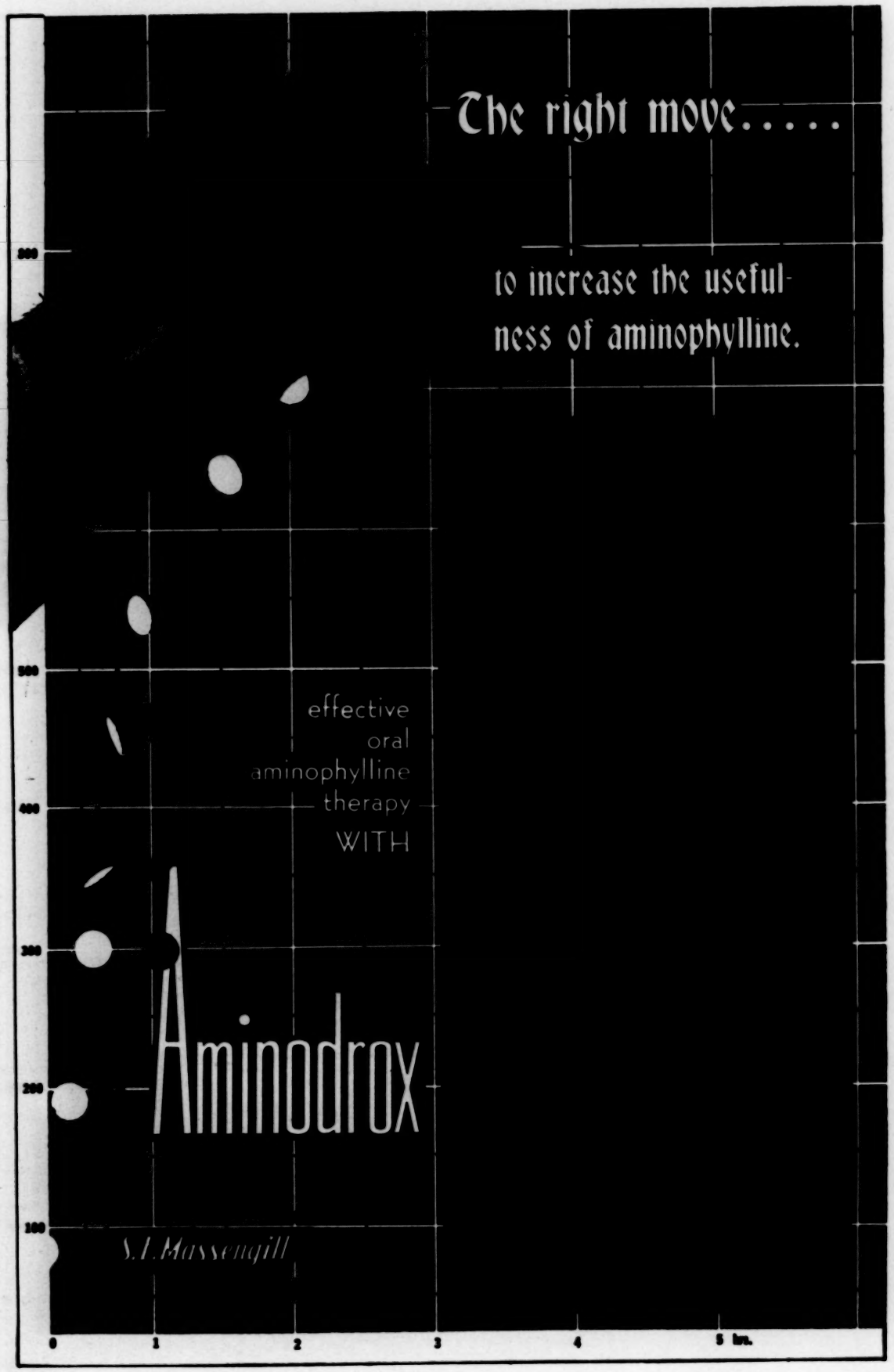
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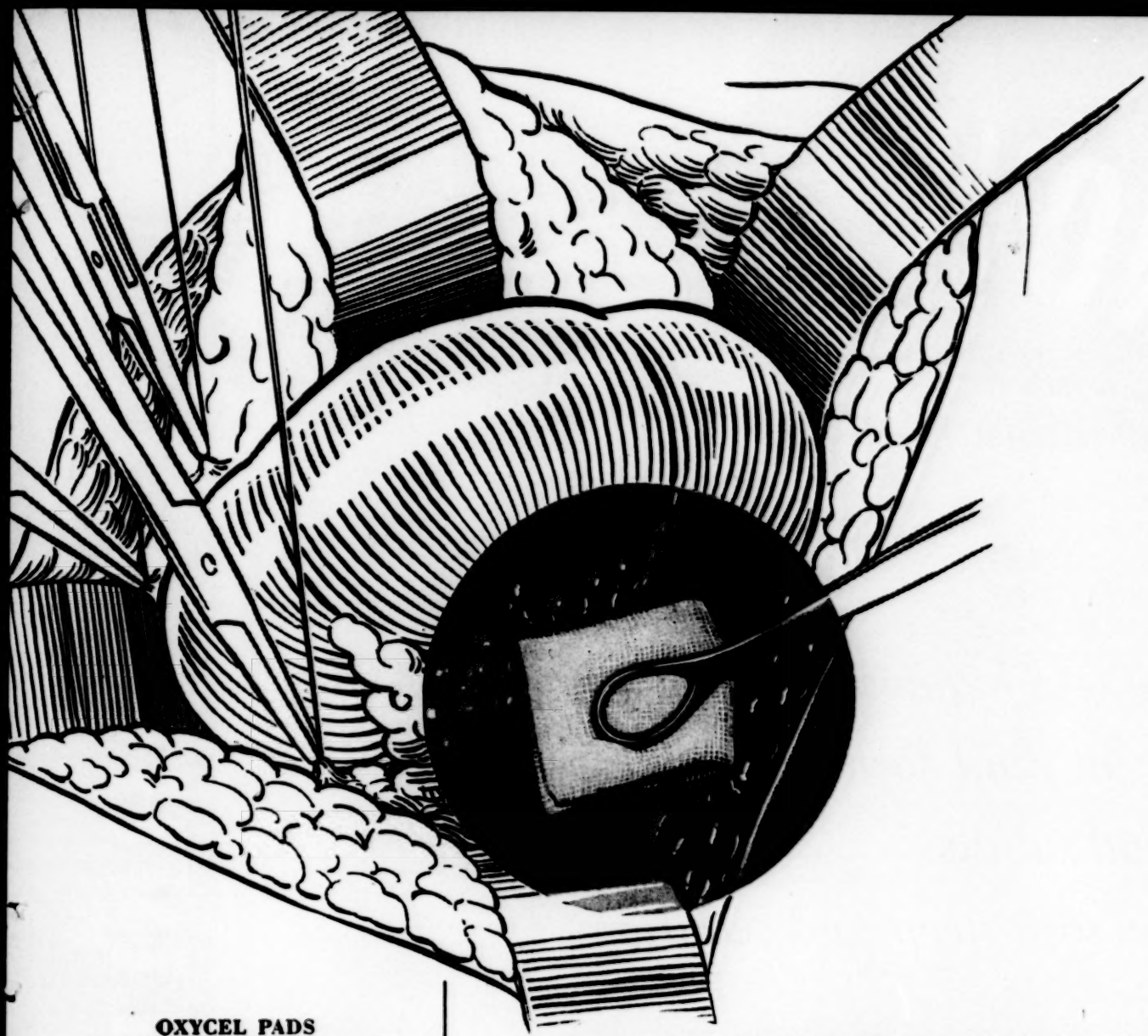

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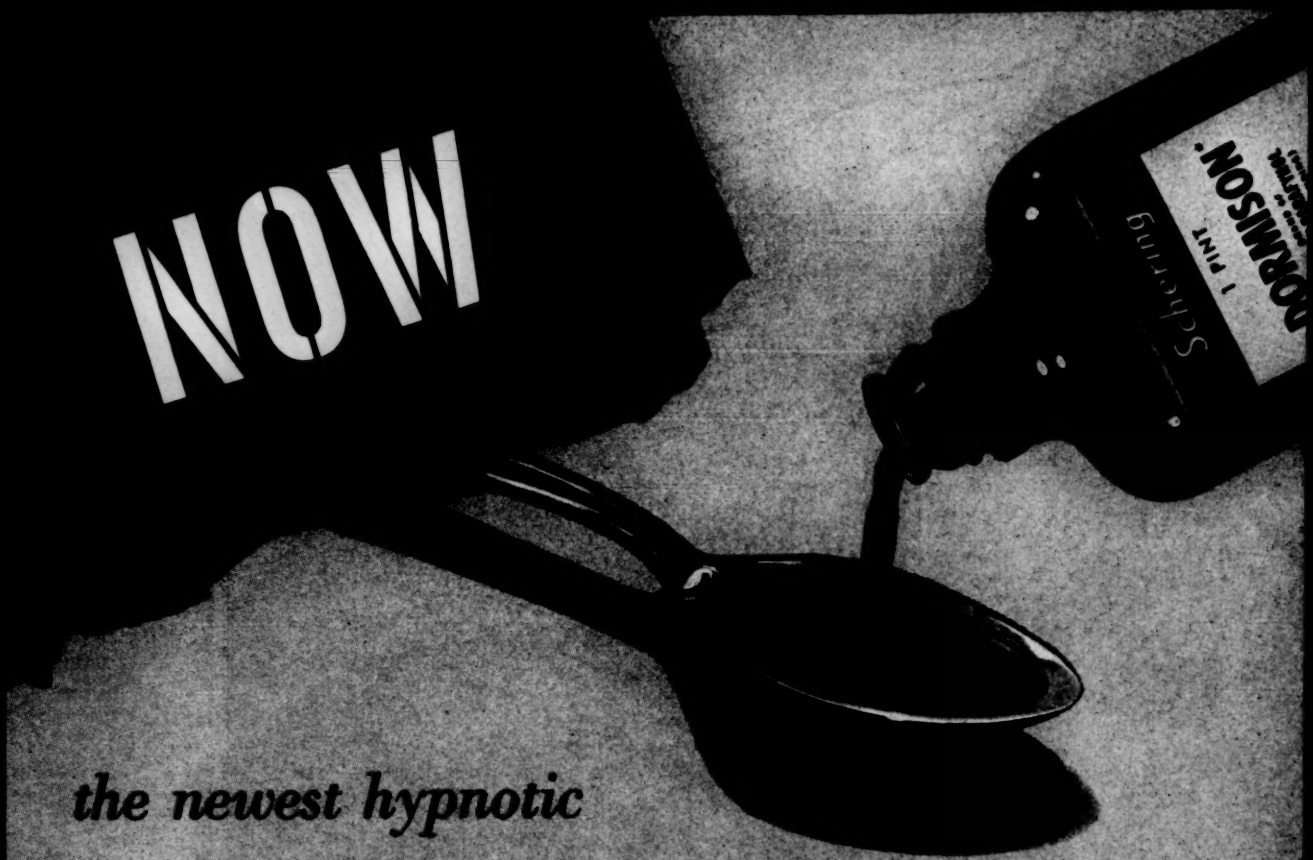

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DORMISON liquid

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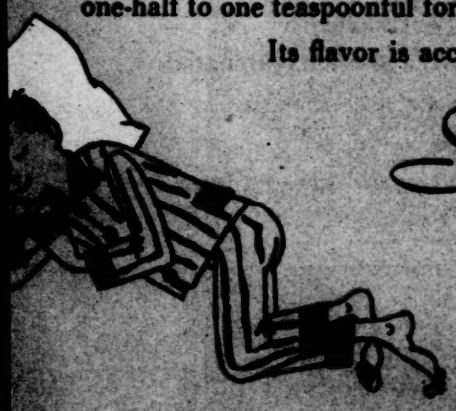
*T.M.



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*1. Barrett, R. H., *The Analeptic Effect of Sodium Succinate on Barbiturate Depression in Man.* Current Researches in Anesthesia and Analgesia. 26: pages 74-81 and 105-113, March-April, May-June, 1947.
2. Greenfield, Irving, *Sodium Succinate as a Test of Circulatory Efficiency.* Ann. Int. Med. 32: 524-527, March 1950.

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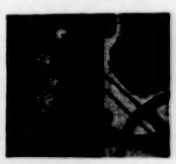

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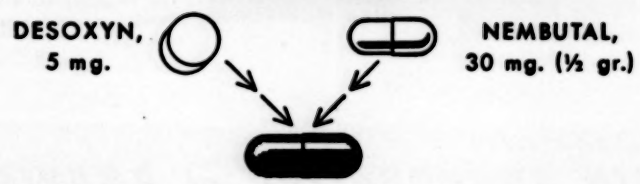

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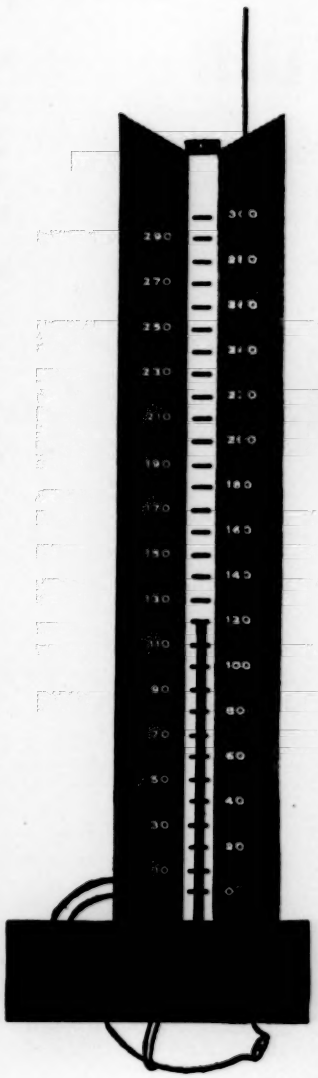

to reduce blood pressure
and relieve
hypertensive symptoms

Methium

TRADEMARK CHLORIDE

(brand of hexamethonium chloride)

a new **oral** *hypotensive*

Blood pressure profoundly altered

Marked reduction in blood pressure in many cases of essential hypertension has been achieved with orally administered Methium. A ganglionic blocking agent which inhibits vasoconstricting impulses through the autonomic nervous system, Methium frequently returns pressure to normal or near normal levels. Extensive use indicates that it may be effective where other therapy has failed.

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Pertenal maintains pressure at more normal levels — eases stress on the heart

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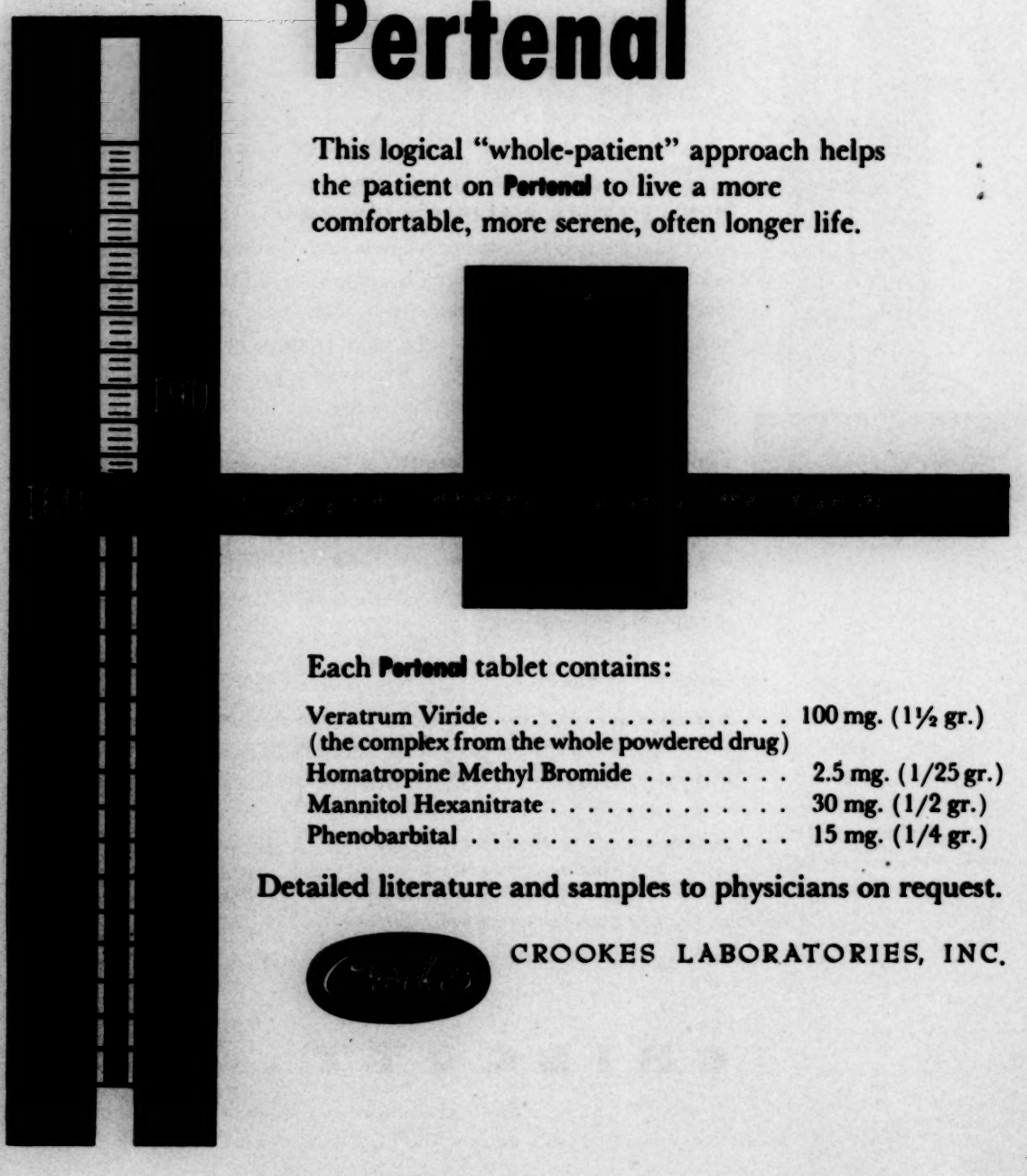

This logical "whole-patient" approach helps the patient on **Pertenal** to live a more comfortable, more serene, often longer life.

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Veratrum Viride (the complex from the whole powdered drug)	100 mg. (1½ gr.)
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Detailed literature and samples to physicians on request.

CROOKES LABORATORIES, INC.

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Mandelamine has the added advantages of being easy to administer and being economical for the patient.

Mandelamine is often preferred in the management of pyelitis, cystitis, pyelonephritis, nonspecific urethritis, prostatitis, and infections associated with neurogenic bladder and urinary calculi, as well as for preoperative and postoperative prophylaxis in urologic surgery.

Supplied: Enteric-coated tablets containing 0.25 Gm. (3¾ gr.) methenamine mandelate — bottles of 120, 500 and 1,000.

Dosage: Adults, 3 or 4 tablets t.i.d.; children, in proportion.

*Mandelamine is the registered trademark of Nepera Chemical Co., Inc., for its brand of methenamine mandelate.

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Octin - *spasmolytic and sympathomimetic*

1... relaxation of smooth muscle spasm especially of the genito-urinary and gastro-intestinal tracts . . . . . 2... in migraine and related headaches.

DOSE: Oral, 1 or 2 Octin tablets, 1 additional tablet in three to four hours.

Intramuscular injection, ½ to 1 cc. Give test dose to determine absence of sensitivity and excessive hypertensive reaction.

Octin ® brand of Isomethoptene, methylisooctenylamine.

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***U. S. PAT. NO. 2,562,830**

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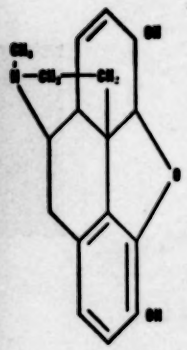

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The American Journal of Medicine

VOL. XIII SEPTEMBER, 1952 No. 3

Editorial

Thermodynamic, Kinetic and Biologic Stability

In normal adult man there is a tendency for the abundances of the various tissue constituents to remain relatively constant or, more precisely, to fluctuate within well defined and often narrow limits. The physician takes cognizance of this generalization every time he orders a quantitative chemical procedure to be performed upon a patient. Whereas the clinician is interested chiefly in the diagnostic significance of values falling outside the limits of normal, the biochemist is more frequently concerned with the mechanisms which may operate to preserve the constancy of composition of normal tissues. In the ensuing analysis of these mechanisms we shall not concern ourselves with the neural and humoral agents that have been described as contributing to homeostasis but shall treat with the question of steadiness of chemical composition at what has been termed the "substrate level."

Thermodynamic Equilibrium. If a system is at the lowest level of free energy attainable at a given temperature, that system is said to be in equilibrium. Regardless of passage of time that system will undergo no spontaneous change since spontaneous change must always involve loss of free energy to the environment. The usual mechanical analogy for this situation is the ball which has rolled to the bottom of the valley. In Figure 1 the ordinates are free energy (F) while the abscissas represent displacement (C). The same point of ultimate arrest will spontaneously be approached, whether from the right or the left, and this is a general characteristic of equilibrium. It will further be noted that the equilibrium point, on these coordinates, is a minimum and, as is always the case with minima, $dF/dC = 0$, meaning that an infinitesimal displacement of the ball to one side or the other entails no change in free energy.

Equilibrium is not merely any state in which no change is apparent. It is, on the contrary, a very particular and precisely defined state *from which no spontaneous change is possible* and is defined simply by the equation:

$$\Delta F = 0$$

Despite the elegance of this definition and the respectability which it has acquired over the years, the term equilibrium is frequently loosely and inaccurately employed. Statements to the contrary notwithstanding, the amino acids of the body are not in equilibrium with body protein. The hydrolysis of protein by water is strongly exergonic and the reaction proceeds essentially to completion, albeit slowly if not catalyzed. Were these nitrogenous constituents truly in equilibrium with each other, one would find essentially all of the nitrogen as amino acid, practically none as protein, whereas in the living organism the situation is almost the opposite. This is an example of one of the many processes which during life may operate steadily although at a point very remote from equilibrium and these processes approach equilibrium only in the autolysis and decay of death. Indeed, the living organism may be differentiated at a biochemical level from the dead one by its capacity, when supplied with exogenous energy, to operate steadily yet in disequilibrium.

Equilibrium does contribute to the chemical steadiness of the organism in certain regards. The body fluids are approximately saturated with respect to the ions of bone salt and the continuous equilibration of plasma with bone mineral tends to preserve the concentration of

these ions at uniform levels in solution. The solution of bone salts is a process which operates close to equilibrium in contrast to the hydrolysis of tissue protein which operates remote from equilibrium.

Kinetic Stability. Essentially all organic compounds release free energy when they react with oxygen to yield CO_2, H_2O and other products. Despite this fact many organic compounds, e.g., glucose, may be stored in the pure state in the presence of oxygen for years without detectable deterioration. This stability, which is clearly not a result of equilibrium, is termed kinetic stability and may be represented by analogy as in Figure 2. The ball has become caught in a crevasse and cannot roll into the valley of equilibrium. If a small amount of work (E) is done to lift the ball over the lip of the crevasse, it can then roll to equilibrium, releasing free energy (ΔF) as it does so.

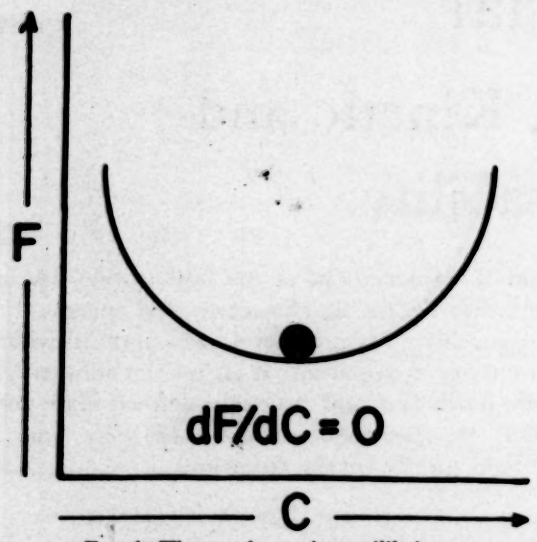

FIG. 1. Thermodynamic equilibrium.

When dealing with a large population of molecules one must extend this analogy somewhat. All molecules are possessed of some thermal energy, as measured by the temperature, and in a kinetic state are continuously colliding and transferring energy from one to another. It must therefore be anticipated that from time to time one molecule will acquire from its neighbors sufficient energy to climb over the energy impediment (E) and roll down the hill. Clearly the rate of a chemical reaction, or the number of molecules which will be excited to roll down the energy hill per unit time, will depend upon the magnitude of the activation energy, E, and the temperature, T. The relationship between these quantities and the velocity constant of a reaction, k, is given by:

$$k = Ae^{-E/RT}$$

where A and R are constants and the exponential $e^{-E/RT}$ is the fraction of all the molecules which at any moment have sufficient energy to exceed the activation energy. If this fraction is very small, as it will be if E is large or if T is small, the reaction velocity becomes very small. Increasing the temperature or decreasing the activation energy will increase the value of $e^{-E/RT}$ and consequently will accelerate the reaction.

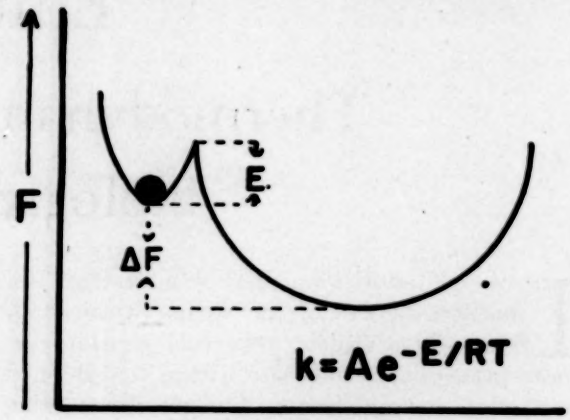

FIG. 2. Kinetic stability.

Appreciable kinetic stability or metastability will be observed in chemical systems of high activation energy, especially at low temperatures. The activation energy for the denaturation of proteins is often extremely high so that, despite the exergonic nature of protein hydrolysis, solutions of proteins such as insulin may often be kept for long periods without appreciable decay, particularly if kept in the refrigerator.

It is the function of enzymes to accelerate chemical reactions and this they do by lowering the activation energy (E). The same glucose which undergoes no detectable decay in the pure state may disappear in a matter of minutes in contact with suitable enzyme preparations such as those of yeast or muscle. Likewise, living cells contain proteolytic enzymes which accelerate markedly the rates of hydrolysis of proteins. For these reasons, substrates in the animal exhibit in general less kinetic stability than do the same compounds in the pure state.

Kinetic stability is, however, a factor to be reckoned with in accounting for the constancy of certain body constituents. Thus collagen is an

extracellular protein which is not only highly insoluble but is also apparently remotely situated from any enzyme capable of activating it. There is evidence that, once deposited, a molecule of collagen has an excellent probability of outliving the animal that harbors it.

Dynamic Steady State. It is clear that neither thermodynamic equilibrium nor kinetic stability will satisfactorily account for all the known examples of constancy of composition of biologic systems since many compounds of the body are known to be in a state of continuous turnover. This implies that whereas the total number of molecules of a given species may remain sensibly constant, a portion of this population of molecules is each day being destroyed or excreted and is simultaneously replaced by new molecules of biosynthetic or dietary origin. The late G. Bernard Shaw, inveterate writer of prefaces, summarized this condition in a preface, written in 1905, to a novel written by him in 1880:[1]

"At present, of course, I am not the author of The Irrational Knot. Physiologists inform us that the substance of our bodies (and consequently of our souls) is shed and renewed at such a rate that no part of us lasts longer than eight years: I am therefore not now in any atom of me the person who wrote The Irrational Knot in 1880. The last of that author perished in 1888; and two of his successors have since joined the majority. Fourth of his line, I cannot be expected to take any very lively interest in the novels of my literary great-grandfather."

It is now known that the fat of liver and of depot, the glycogen of muscle and liver, the several proteins of plasma and internal organs, the glucose and cholesterol of the blood, as well as numerous other tissue components undergo continuous turnover in the adult animal in balance, each at its own peculiar rate. The mechanical analogy is indicated in Figure 3, wherein balls are shown to be continuously rolling down the energy hill only to be replaced by new balls that have been pumped up to a higher energy level by some separate process. The number of molecules at the peak of the energy curve is reasonably constant only if the rate at which new molecules are being generated is matched by the rate at which old molecules decay. Any imbalance in these two rates will result in a change in composition and, in general, it is to such an imbalance that abnormal analytic values determined in the clinical laboratory must be attributed. A rise above normal limits in the concentration of glucose in the blood is, therefore, a reflection either of an increase in the rate at which glucose enters the blood, or of a decrease in the rate at which it is removed from the blood, or a combination of these two disturbances.

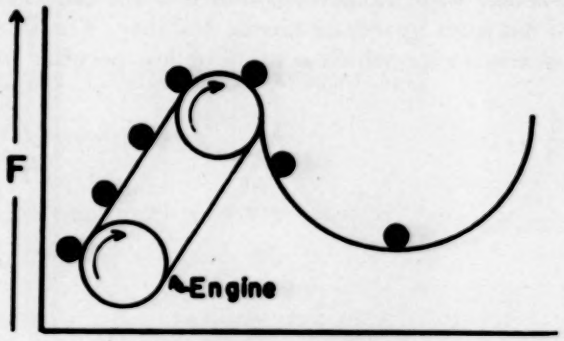

FIG. 3. Dynamic steady state.

Many metabolites are formed in the body by processes which consume energy. The continuous regeneration of these molecules requires the operation of an engine and the immediate fuel for this engine appears to be, in many cases, the chemical energy of adenosine triphosphate and other phosphate compounds. The ultimate energy source is, of course, solar radiation which during photosynthesis exalts electrons from the low energy level of water to the higher energy levels found in organic foodstuffs. During the respiratory phase of mammalian metabolism energy-rich electrons of the nutrient cascade downward by a series of steps until they are returned to the low energy level of water and, coupled with the energy released by this cascade, energy-rich phosphate compounds, the immediate fuel of the engine, are formed.

What happens when the engine fails? Such balls (Fig. 3) as are at energy levels above that of equilibrium will continue to roll down the hill. This decline in energy level may be slow insofar as there may be steps at which activation energy is required. Inevitably, however, the lowest possible level of free energy, the equilibrium point, will be attained. With the engine no longer operative, no balls will be hoisted to the high energy level and the system runs down. This is a picture of the dead, not of the living organism.

Final assessment of the contributions to biochemical stability of the three factors which have been discussed can certainly not be made

[1] SHAW, G. B. The Irrational Knot. New York, 1905. Brentano's.

at this time. The role of thermodynamic equilibrium is limited by virtue of the fact that so many biologic processes are adjusted to operate at points remote from the equilibrium point. Kinetic stability in a biologic system undoubtedly contributes to the constancy of chemical composition with the proviso that it is the function of enzymes to reduce kinetic stability. The stabilizing factor which is more or less peculiar to living systems is the dynamic steady state in which energy-linked synthetic processes offset spontaneous degradative processes to maintain, in the normal animal, the required degree of constancy of chemical composition.

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Clinical Studies

Parahemophilia in Three Siblings (Owren's Disease)*

With Studies on Certain Plasma Components Affecting Prothrombin Conversion

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ALTHOUGH the importance of plasma and serum constituents in prothrombin conversion is well recognized, the relationship between the numerous entities described by many observers is still obscure. Rapid thrombin formation requires the so-called "labile factor" (L.F.), deterioration of which explains the retarded prothrombin conversion of aged plasma.[1,2] Ware and colleagues,[3,4] and Fantl and Nance[5] also have demonstrated the existence of non-prothrombin plasma factors which affect the velocity of prothrombin conversion, referring to their substances as the plasma accelerator globulin (Ac-globulin) and "prothrombin accelerator," respectively.

The pathologic and clinical significance of inadequacy of such an entity is becoming increasingly evident. Sykes and associates[6] have described plasma Ac-globulin deficiency in experimentally induced liver poisoning, and Alexander and Goldstein[7] found Ac-globulin and L.F. deficiency in severe hepatic disease in man. Similarly, Owren[8,9] presented an exhaustive clinical and laboratory study of a unique patient congenitally deficient in a plasma constituent essential for rapid thrombin formation which he originally termed "factor V" and later renamed "proaccelerin."[10] The hemorrhagic diathesis associated with this defect was termed "parahemophilia" because the elevated clotting time simulated that observed in hemophilia. Additional cases have recently been reported by de Vries et al.[11]

It is highly unlikely that these entities represent different substances. It would be more reasonable to assume that they are identical or closely related and that reported differences are only apparent, reflecting differences in technics of study, in species and in interpretations. However, definite proof regarding the identity of all these moieties is still lacking.

The purpose of this article is to present observations on three siblings with a clotting defect indistinguishable from Owren's original case of parahemophilia. The data warrant the conclusion that L.F., the factor of Fantl and Nance, plasma Ac-globulin and factor V are all identical. The information also indicates the unequivocal difference between this factor and the prothrombin conversion accelerator found in serum (SPCA).[12,13,14]

CASE REPORTS

M. G. (M-17221), a seventeen year old white school girl of Italian extraction, was admitted on December 26, 1950, having been referred by Dr. Ralph Gancher of Waterbury, Connecticut, with a diagnosis of idiopathic hypoprothrombinemia. Hemorrhagic phenomena were first noted at the age of five following a tonsillectomy, requiring transfusion. Thereafter the patient experienced many bleeding episodes: innumerable epistaxes, easy bruising, severe hemorrhages following tooth extractions, severe bleeding after an eyebrow cyst was lanced and profuse menorrhagia. She had also noted persistent bleeding following cuts, lacerations and con-

* From the Yamins Research Laboratory, Beth Israel Hospital, and the Department of Medicine, Harvard Medical School, Boston, Mass. Supported by a grant from the United States Public Health Service and an institutional grant to Harvard University from the American Cancer Society.

tusions, and ease of fatigue, dyspnea on exertion and occasional giddiness. The patient had had no joint, pulmonary, gastrointestinal or urinary tract bleeding, and her past history was otherwise non-contributory. There was no history of a hemorrhagic diathesis in the family; the or adenopathy. The heart and lungs were normal, as was the abdomen. Liver and spleen were not palpable or enlarged to percussion. Extremities were normal. Blood pressure was 118/62; capillary fragility was normal.

Routine tests revealed the urine to be nega-

TABLE I

SALIENT CHARACTERISTICS OF PARAHEMOPHILIA IN THREE SIBLINGS

	Subject		
	M. G., 0+	A. G., 0+	J. G., 0→
Age	17 yr.	15 yr.	4 yr.
Hemorrhagic phenomena	+++	±	0
Other defects	0	+*	0
Capillary fragility	Normal	.....	.....
Platelets	230,000 per cu. mm.	Abundant on smear	Abundant on smear
Bleeding time (min.)	24–32+ (2–5)†	24	25+
Clotting time (min.)	39–45 (6–14)†	36	31
Clot retraction	Normal	Normal	Normal
Prothrombin time (sec.)			
Whole plasma	44–67 (15–16)†	43–48	45
Dilution technic	33–46 (26–30)†	35–39	43
Two-stage prothrombin (units/ml.)			
Without Ac-globulin supplement	Too small to measure	Too small to measure	Too small to measure
With Ac-globulin supplement	100–188	141–168	180
Ac-globulin (per cent of normal)	5	5	5
Serum prothrombin (units/ml.)			
20′ after blood shed	150	.....	.....
40′ after blood shed	135	168	.....
60′ after blood shed	98	125	78
SPCA elaboration	Normal	Normal	Normal

* Epidermolysis bullosa congenitalis.

† Values in parentheses represent the normal values obtained in our laboratories.

mother and father are living and well, as are several aunts and uncles.

Two years prior to admission the patient had been studied at the Waterbury Hospital where a diagnosis of idiopathic hypoprothrombinemia was made. At that time the bleeding and clotting times were reported to be five minutes, respectively, and the plasma prothrombin was said to be 10 per cent of normal. Plasma protein concentration, fibrinogen, blood ascorbic acid, platelet count and marrow were described as normal. For one year prior to admission the patient had received injections of vitamin K and liver extract weekly with no beneficial effect.

Physical examination was not remarkable except for moderate pallor and a few small generalized ecchymoses. There was no icterus

tive, red blood count 3.1 million, hemoglobin 7 gm. per cent, white blood count 4,700, with a normal differential. Hematocrit, 25 per cent packed cells. Platelet count was 230,000 per cu. mm. The clotting time (Lee-White at 37°C.) was repeatedly elevated to approximately forty-five minutes (normal eight to fifteen minutes); bleeding time (Duke) exceeded thirty-two minutes; clot retraction normal. Blood group was A, Rh negative. Blood bilirubin was 0.6 mg. per cent: total protein, 7.3 gm. per cent; albumin 4.9 gm. per cent; globulin 2.4 gm. per cent; thymol turbidity, 5.6 units; cephalin flocculation test 2+; Hinton test was negative.

The patient was readmitted on July 10, 1951, for extraction of two carious teeth. The findings were essentially unchanged; clotting time,

39 minutes; bleeding time, 24.5 minutes; clot retraction normal; capillary fragility normal. Profound anemia was again observed.

The two siblings had never experienced frank hemorrhagic episodes although they showed the same clotting defect and elevated bleeding time. A. G., a fifteen year old sister, suffered from epidermolysis bullosa congenitalis* but was otherwise well, as was the four year old brother, J. G. The blood of A. G. and J. G. were type A and B, respectively, both Rh positive. Blood from the parents showed no clotting abnormalities.

TABLE II
PLASMA PROTHROMBIC ACTIVITY IN PARAHEMOPHILIA

Date	One-Stage				Two-Stage	
	Whole Plasma		1:10 Dilution with $BaSO_4$ Normal Plasma		Without Ac-globulin Supplement (units per ml. of plasma)	With Ac-globulin Supplement (units per ml. of plasma)
	Prothrombin Time (sec.)	Prothrombin Activity (% of normal)	Prothrombin Time (sec.)	Prothrombin Activity (% of normal)		
Patient M. G.						
12/26/50	67(15)*	2	38(30)*	54	<5	122
12/27/50	59(15)	3	34(26)	70	<5	185
12/28/50	55(16)	3	33(28)	68	<5	188
7/10/51	58(15)	3	41(30)	52	<5	120
7/13/51	54(16)	3	45(29)	45	..	106
7/17/51	54(−)	3	46(−)	45	..	100
7/23/51	44(15)	5	43(30)	50	<5	156
Patient A. G.						
7/10/51	43(15)	5	35(30)	68	<5	141
7/23/51	48(15)	5	37(30)	57	<5	168
Subject J. G.						
7/23/51	45(15)	5	43(30)	50	<5	180
Patients M. G., A. G., J. G. Plasmas from Each Mixed in Equiproportion						
7/23/51	44(15)	5	.....	..	..	..

* Figures in parentheses denote prothrombin times on normal plasma obtained concurrently under the same conditions. The prothrombin time of the $BaSO_4$ adsorbed normal plasma was greater than 180 seconds. Ac-globulin supplement in the two-stage system consisted of bovine $BaCO_3$ adsorbed serum (1:150 dilution) according to the method of Ware and Seegers.[18] Two-stage prothrombin values of normal plasma in our laboratories are 200–300 units per ml.

* To exclude the possibility that this skin disorder is associated with a coagulation defect, clotting studies were performed on another patient with this disease; no abnormality was detected.

The results of the laboratory investigations on the patient and her siblings are recorded hereinafter. A description of the methods and materials employed is presented with each experiment.

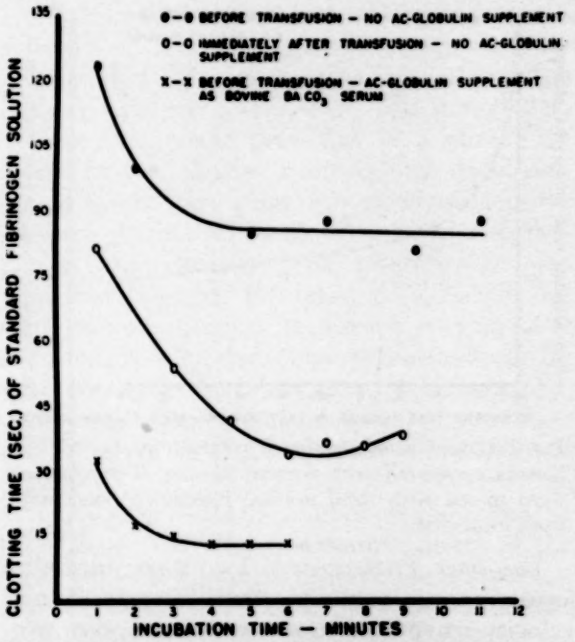


FIG. 1. Two-stage prothrombin conversion in parahemophiliac M. G. Ac-globulin supplemented as a 1:150 dilution of bovine $BaCO_3$ adsorbed serum.[18]

RESULTS

The salient clinical and laboratory features of our cases of parahemophilia are presented in Table I.

Prothrombic Activity—One Stage. The prothrombin time of the undiluted plasma of the three subjects, determined by the orthodox procedure of Quick[15] employing rabbit brain thromboplastin (difco®), was found repeatedly elevated to between 45 and 67 seconds. (Table I.) These values represent approximately 2 to 5 per cent of normal prothrombic activity. By the modified procedure[16] in which the test plasma is diluted with normal plasma deprothrombinated by $BaSO_4$ adsorption,* the prothrombic activity was 44 to 70 per cent of normal. (Table II.) The same results were obtained when human brain thromboplastin (acetone dehydrated) was substituted for rabbit brain.

Comment: The prothrombin times of the whole

* Hereafter referred to as "$BaSO_4$ plasma." It should be pointed out that preparations of $BaSO_4$ may differ in their adsorbing capacity. Thus $BaSO_4$ Baker has tenfold the capacity of $BaSO_4$ Merck. We use Baker $BaSO_4$ (C. P.) exclusively.

oxalated plasma in parahemophilia were markedly elevated but this was rectified by the admixture of deprothrombinated normal plasma. Mild to moderate hypoprothrombinemia was also evident.

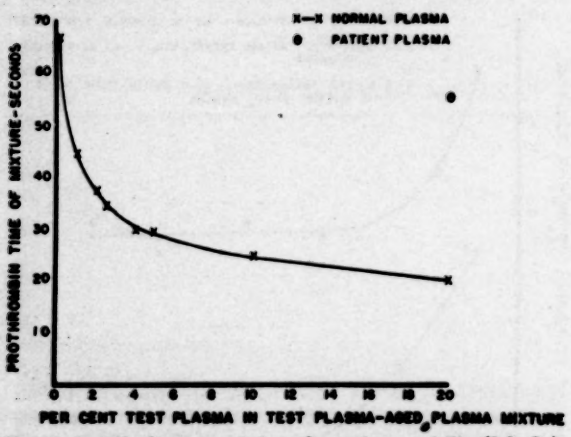


FIG. 2. Labile factor activity of parahemophilic (M. G.) plasma compared with normal plasma. Test plasmas were mixed with aged normal plasma in concentrations indicated.

Two-stage Prothrombin. Two-stage thrombin formation was negligible both in quantity and velocity when measured by the orthodox two-stage method (without Ac-globulin supplement).[17] In striking contrast substantial prothrombin conversion occurred with the modified procedure[18] in which $BaCO_3$ adsorbed bovine serum is provided as a source of optimal amounts of Ac-globulin. (Table II, Fig. 1.) In these experiments bovine lung thromboplastin was used.

Comment: Two-stage prothrombin conversion was negligible unless a non-prothrombin factor (Ac-globulin) was supplemented. Under the latter conditions, also, some degree of hypoprothrombinemia was demonstrable, comparable to the one-stage values.

Labile Factor Activity. L.F. is measurable by its ability to rectify the retarded prothrombin conversion of aged normal plasma.[19] The activity of parahemophilic plasma was extremely low in this regard. Whereas one part of normal plasma added to four of aged plasma lowered the prothrombin time from 69 seconds to 21 seconds, M. G.'s plasma could lower the prothrombin time only to 57 seconds. (Fig. 2.) On another occasion plasma from M. G., A. G. and J. G. could lower an aged plasma prothrombin time only from 95 seconds to 66, 54 and 60 seconds, respectively.

Comment: L.F. activity in three parahemophiliacs was less than 5 per cent of normal.

Ac-globulin Activity. Ac-globulin is required for the rapid conversion of prothrombin to thrombin by thromboplastin and calcium in the isolated two-stage system.[4] The retarded and incomplete prothrombin conversion in parahemophilia by the orthodox two-stage method and its rectification by $BaCO_3$ bovine serum (Fig. 1) suggested Ac-globulin deficiency. This was substantiated by the inability of parahemophilic plasma to accelerate thrombin formation in a mixture containing purified human prothrombin, thromboplastin and calcium. Prothrombin was separated from normal plasma and purified by $BaSO_4$ adsorption, elution with

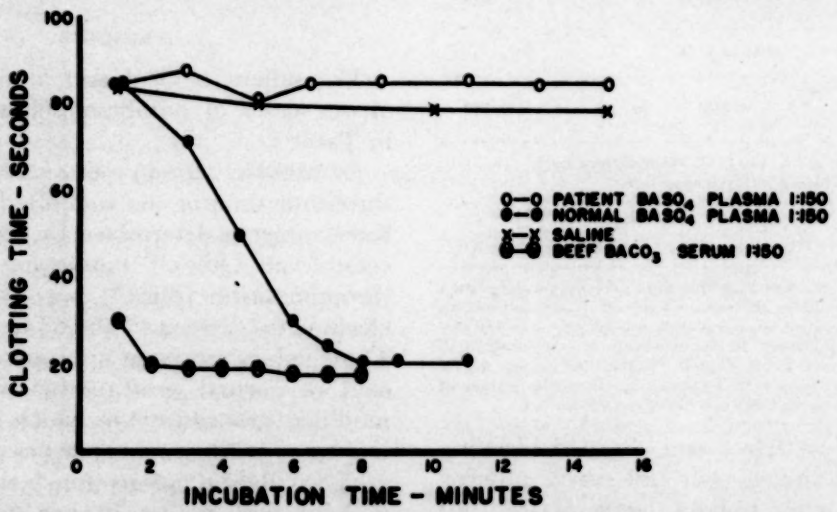


FIG. 3. Ac-globulin activity of parahemophilic (M. G.) plasma on thrombin formation from purified human prothrombin. Reaction mixture contains purified prothrombin, thromboplastin, calcium plus the agents indicated.

sodium citrate, and dialysis, according to a method previously described.[14] In the presence of calcium and bovine lung thromboplastin, thrombin evolved from the prothrombin extremely slowly. (Fig. 3.) Addition of the parahemophilic deprothrombinated ($BaSO_4$) plasma failed to influence prothrombin conversion appreciably, in marked contrast to the effect of normal $BaSO_4$ plasma.

TABLE III
EFFECT OF FACTOR V ON CLOTTING DEFECT OF PARAHEMOPHILIC BLOOD (M. G.) AND AGED NORMAL PLASMA

Plasma Mixture (Parts)					Prothrombin Time (sec.)
Patient Plasma	Normal Plasma	Normal Seitz Plasma	Normal $BaSO_4$ Plasma	Normal Aged (14 Day) Plasma	
1	—	—	—	—	62
—	1	—	—	—	16
—	—	1	—	—	>180
—	—	—	1	—	>180
1	—	9	—	—	41
1	—	—	9	—	36
—	1	9	—	—	30
—	1	—	9	—	29
1	—	—	—	—	56
—	—	1	—	—	>300
—	—	—	1	—	>300
—	—	—	—	1	86
—	1	—	—	—	16
1	—	9	—	—	45
1	—	—	9	—	41
—	—	1	—	4	20
—	1	—	—	4	18
—	—	—	1	4	18
—	1	9	—	—	30
—	1	—	9	—	29

Comment: These observations, in agreement with the rectifying effect of normal $BaSO_4$ plasma in the one-stage system, provide indisputable evidence that the patient's plasma was relatively devoid of Ac-globulin activity.

Factor V Activity. In Owren's original case the elevated prothrombin time could be corrected by the addition of normal plasma deprothrombinated by Seitz filtration. Accordingly, 35 ml. of normal oxalated plasma were passed through a Seitz filter (Hercules type S, size 13, diameter 3.6 cm.). The first 5 ml. were discarded and the remainder was refiltered through the same pad. The filtrate, prothrombin-free, contained L.F. and Ac-globulin activity, and could correct the clotting defect of parahemophiliac M. G. (Table III.) It was, moreover, practically identical with normal $BaSO_4$ plasma in these respects.

Comment: The patient was lacking in factor V activity.

Study of Purified Prothrombin Derived from the Pathologic Plasma. This study was intended to exclude the remote possibility of a qualitative defect in the plasma prothrombin itself, viz., that it might have been only slowly susceptible for some obscure reason to the action of thromboplastin and calcium. The prothrombin was separated from M. G.'s plasma by $BaSO_4$ adsorption and elution with citrate. As was to be expected, the fraction behaved normally as far as its convertibility to thrombin was concerned; it required supplements of Ac-globulin as does prothrombin similarly derived from normal plasma.

Antithrombic Activity. Although there can be little doubt that the coagulation defect of the three subjects centered around the conversion of prothrombin to thrombin, it seemed desirable to rule out excessive plasma antithrombic activity. By the technic of Klein and Seegers[20] the antithrombic activity was found normal in patient M. G. (Fig. 4.)

*Prothrombin Consumption.** Since in the isolated system Ac-globulin deficiency is said to result in poor yields of thrombin as well as retarded thrombin evolution,[4,21] it was deemed important to study prothrombin consumption during the course of spontaneous coagulation. Accordingly, the following experiments were performed: aliquot samples (2.1 ml.) of freshly drawn blood were placed in separate uncoated clean dry test tubes kept at 37°c. At measured intervals 0.1 M sodium oxalate (1 to 9 vols. of blood) was added in order to stop the clotting process. Mixtures were gently stirred with a glass rod, the sera were separated and incubated for fifteen minutes in order to inactivate thrombin, and they were then analyzed for prothrom-

* A few words of caution are indicated. The term "prothrombin consumption" is widely used to describe the disappearance of prothrombin during coagulation, the implication being that the prothrombin is all converted to thrombin. This is not completely justified until it can be shown that the thrombin formed is equal to the amount of prothrombin which vanishes. Other ways in which prothrombin may disappear must always be considered.

bic activity by both the modified one- and two-stage procedures.

In M. G. prothrombin conversion was markedly retarded (Figs. 5, 6) but the prothrombin completely disappeared if sufficient time was allotted. Similar results were obtained in A. G. and J. G.: sixty minutes after the blood was shed, residual serum prothrombin was 125 and 75 units per ml., respectively, values which are definitely above normal. Prothrombin disappearance (prothrombin consumption) was accelerated by blood transfusion. (Fig. 5.)

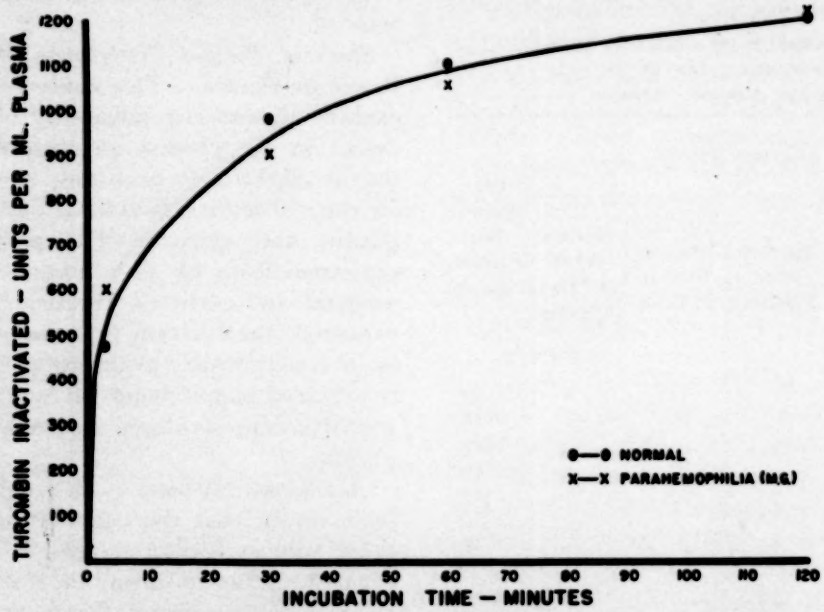


FIG. 4. Antithrombic activity of parahemophiliac M. G.; 1.0 ml. of thrombin solution (Cutter Lab., human thrombin) containing 1,400 units per ml. was added to 1.0 ml. of plasma, previously defibrinated by small amounts of thrombin (1 unit per ml. of oxalated plasma). At intervals specified, an aliquot of the thrombin-plasma mixture was removed, suitably diluted with saline, and then measured for residual thrombic activity on a standard fibrinogen solution, according to the method of Klein and Seegers.[20]

Platelet Agglutination. Little is known regarding the factors which induce platelet agglutination and lysis during spontaneous coagulation. Normally, this begins within a few minutes after blood is exposed to a "foreign" surface (glass). Under certain pathologic (hemophilia, circulating anticoagulant) or experimental (prompt decalcification and siliconization of surfaces) conditions, platelet agglutination is retarded. According to Quick and colleagues[22] evolved thrombin is responsible for these changes.

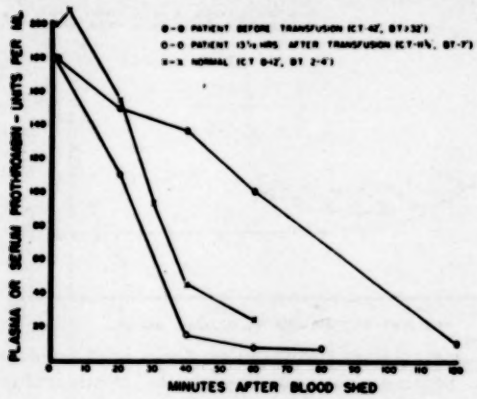
This phenomenon was studied in order to see whether it was affected by retarded thrombin evolution consequent to Ac-globulin deficiency. Accordingly, blood was drawn with a siliconized syringe and arquad* coated needle. At measured intervals coagulation was stopped by the addition of citrate (1 vol. of 2.5 per cent sodium citrate to 9 vols. of blood), and the platelets were visualized and counted by direct microscopy, using the Rees-Ecker procedure. Platelet agglutination was found considerably retarded, along with over-all coagulation and prothrombin conversion, compared with a normal blood handled the same way. (Fig. 6.)

"Plasma" Ac-globulin, "Serum" Ac-globulin Relationship. According to Ware and Seegers, *plasma* Ac-globulin is the relatively inert precursor of *serum* Ac-globulin to which it is converted by thrombin evolving during the coagulation process. The conversion can also be induced by the addition of thrombin to whole plasma or to purified *plasma* Ac-globulin. We have observed similarly that thrombin added to whole normal, or to $BaSO_4$ normal plasma which contains full amounts of L.F. and plasma Ac-globulin, induces markedly increased Ac-globulin and L.F. activity.

It was of interest to compare the effect of adding thrombin to parahemophilic plasma.

* A non-wetting agent generously provided by Armour Co., Chicago, Ill.

Accordingly, human thrombin was added to M. G.'s plasma and to normal plasma (2.2 units per ml.). The resulting fibrin was removed, the supernatant was added after variable intervals of time to aged normal plasma and the prothrombin times of the mixtures were determined. portions studied the pathologic plasma was indistinguishable from aged normal plasma insofar as the rectifying effect of fresh normal plasma is concerned. It is also noteworthy that the clot-promoting effect of the normal plasma is attributable to a plasma component not adsorb-



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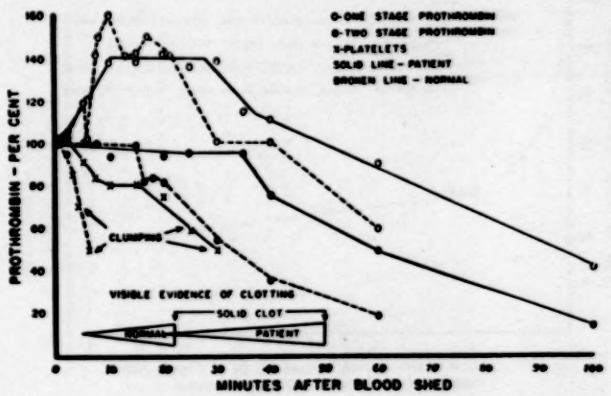

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FIG. 5. Prothrombin consumption, before and after transfusion, in parahemophiliac M. G. Prothrombin in plasma and serum determined by modified two-stage method in which optimal Ac-globulin is provided by admixture of bovine $BaCO_3$ serum.[18]

FIG. 6. Changes in one-stage and two-stage prothrombic activity, platelet number and clumping, and visible fibrin deposition in freshly shed parahemophilic (M. G.) blood compared with normal blood. On the ordinate is also plotted the platelet number (in per cent of the original which is 100 per cent).

(Table IV.) The normal thrombin-treated plasma had a greater restorative effect on aged plasma than did the normal plasma alone (15.4 compared with 20 seconds, a difference which in this range reflects a profound enhancement in L.F. activity). In contrast, the effect of thrombin-treated parahemophilic plasma was practically the same as the untreated plasma (51 compared with 59.5 seconds) a change which in this range of the curve (Fig. 2) represents a negligible alteration in over-all L.F. activity). This further supports the conclusion that the pathologic plasma was deficient in Ac-globulin, or that its Ac-globulin could not be converted to the active "serum type" by thrombin.

Quantitative Relationships between Ac-globulin and Prothrombic Activity. It has already been shown that fresh parahemophilic plasma cannot correct the retarded prothrombin conversion of aged plasma. This suggests that the defect of the pathologic plasma is identical with that induced in normal plasma by aging. In accordance with this interpretation are the observations in Figure 7 which show the prothrombin times of varying mixtures of (1) normal plasma with aged normal plasma, and (2) normal plasma with fresh parahemophilic plasma. In all pro-

TABLE IV

LABILE FACTOR ACTIVITY OF NORMAL OR PARAHEMOPHILIC PLASMA TREATED WITH THROMBIN *

Plasma Mixture	Prothrombin Time (sec.)	
	Normal	Parahemophiliac M. G.
Aged plasma + saline alone	70	....
Aged plasma + fresh plasma	20	59.5
Aged plasma + thrombin-treated plasma		
10 min. incubated	15.6	56.8
30 min. incubated	15.4	51.0
60 min. incubated	16.9	55.7

* Prothrombin times determined on plasma mixtures comprising 0.4 ml. of aged normal oxalated plasma plus 0.1 ml. of thrombin-treated or non-treated plasma.

2.2 units of thrombin (Cutter Laboratory, human thrombin) in 0.1 ml. of physiologic saline were added to 1.0 ml. of the plasmas. The combinations were incubated (room temperature) for 10, 30, and 60 minutes, respectively, and 0.1 ml. of the combinations were then added to 0.4 ml. of the aged plasma.

able by $BaSO_4$, a fact which excludes both prothrombin and the precursor of SPCA.

It can now be assumed that L.F., plasma Ac-globulin and factor V are one entity. Its concentration is critically low in humans,[23,24] far lower than in bovine, canine or rabbit plasma.

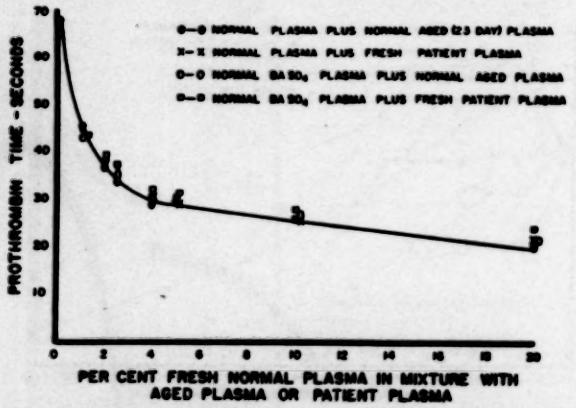


FIG. 7. Rectifying effect of normal plasma on the retarded prothrombin conversion of fresh parahemophilic plasma (M. G.) or of aged normal plasma.

When reduced to 50 per cent or less of its normal value in man, prothrombin conversion becomes retarded. The availability of plasma congenitally devoid of this factor provided an excellent opportunity to re-examine more precisely the quantitative relationships between its concentration and the velocity of thrombin elaboration. This was done by determining the prothrombin times of mixtures of normal plasma with the pathologic plasma in various proportions and under varying conditions.

As Ac-globulin is decreased below approximately 70 per cent of normal, the curve relating prothrombin time with prothrombin concentration progressively deviates from that obtained when Ac-globulin is maintained at 100 per cent. (Fig. 8.) Retardation in thrombin evolution becomes progressively marked as the Ac-globulin falls below 20 per cent.

Since in this experiment the concentrations of both prothrombin and Ac-globulin varied, it seemed desirable to setup conditions in which the Ac-globulin was the only variable. This was accomplished by mixing 0.4 ml. of the abnormal plasma with 0.1 ml. of saline containing variable amounts of normal $BaSO_4$ plasma. The data obtained under these circumstances (Fig. 9) also indicate a similar relationship between Ac-globulin concentration and the prothrombin time at prothrombin levels maintained between 50 to 54 per cent of normal. It is of interest that the configuration of the curve relating Ac-globulin concentration and prothrombin time resembles that relating prothrombin level with the prothrombin time.

Additional pertinent information was provided by the following experiment: Normal

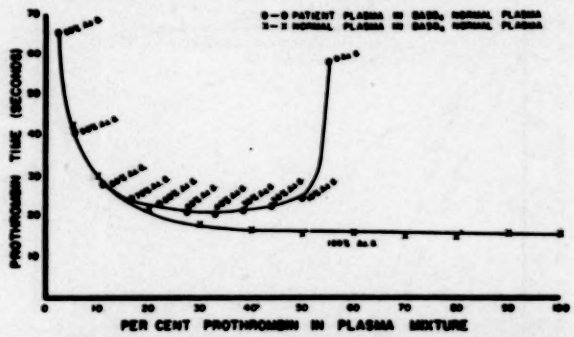


FIG. 8. Curve relating prothrombin times with concentrations of prothrombin and Ac-globulin. Prothrombin concentrations in mixtures were calculated from two-stage prothrombin determinations on the normal plasma and on the parahemophilic (M. G.) plasma. Ac-globulin concentrations were calculated on the basis that the whole normal plasma and $BaSO_4$ normal plasma contained 100 per cent Ac-globulin. The parahemophilic plasma was assumed to contain no Ac-globulin.

plasma was mixed in various proportions with $BaSO_4$ adsorbed parahemophilic plasma or with $BaSO_4$ normal plasma. In the latter mixtures Ac-globulin was 100 per cent since $BaSO_4$ does not absorb this clotting factor, whereas in the normal parahemophilic mixtures Ac-globulin is furnished almost entirely by the normal plasma contained therein. Again, the discrepancies in prothrombin times (Fig. 10) reflect the profoundly retarded prothrombin conversion caused by the limitation of Ac-globulin.

Study of the SPCA Mechanism. Considerable evidence is already available indicating that SPCA is distinct from Ac-globulin, L.F. and factor V.[12–14] A hemorrhagic diathesis simulating hypoprothrombinemia in a patient with congenital inadequacy of SPCA has been described.[25] This subject's plasma had normal amounts of Ac-globulin but showed poor SPCA elaboration.

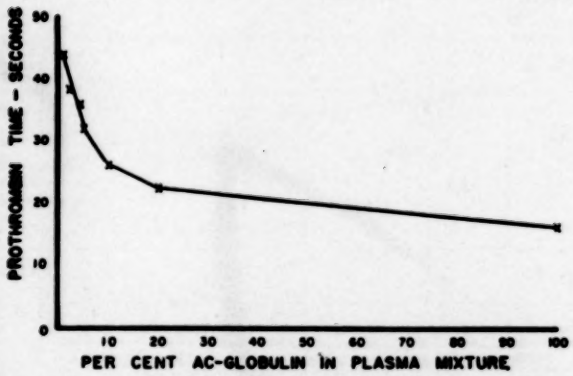
In parahemophilia SPCA formation from its precursor in plasma is normal despite the retarded prothrombin consumption and negligible Ac-globulin concentration. (Table v.) Furthermore, in the one patient thus studied (M. G.) the serum showed SPCA activity even when tested on the parent plasma. These observations provide substantial evidence that SPCA does

not evolve from Ac-globulin. Moreover, normal Ac-globulin activity is not required for SPCA formation.

The aforementioned conclusions are supported by additional data obtained on aging plasma. When normal plasma is stored at refrigerator temperatures, Ac-globulin deteriorates and SPCA evolves.[26] Whereas the prothrombin time of the whole plasma rises, there is progressive shortening of the prothrombin time when it is determined by a technic wherein normal amounts of Ac-globulin are provided to replace that lost by deterioration. Similar observations are found in parahemophilic plasma during aging: the originally elevated prothrombin time of the whole plasma increases even more, but prothrombic hyperactivity develops (Fig. 11) just as it does in normal plasma.

The elaboration of SPCA also shows itself through the development of prothrombic hyperactivity (one stage) early in the course of spontaneous coagulation, frequently before there is any detectable evidence of prothrombin disappearance as measured by the two-stage procedure.[27] Indeed, this phenomenon has long been used by us to detect "chemical coagulation" before there is visible evidence of fibrin deposition, and has been employed to evaluate the efficacy of various procedures designed to inhibit the clotting process. The data in Figure 6 indicate that such prothrombic hyperactivity developed in parahemophilic M. G. early in the course of spontaneous coagulation just as does normal blood.

Comment: Demonstration of a normal SPCA mechanism in parahemophilia establishes SPCA and its precursor as entities distinct from Ac-globulin. Furthermore, Ac-globulin appears to play no role in SPCA elaboration.



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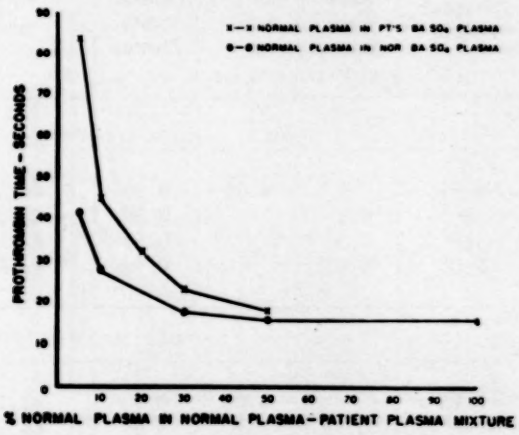

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FIG. 9. Curve relating prothrombin times with varying concentrations of Ac-globulin in plasma mixtures containing a relatively fixed prothrombin concentration (50 to 54 per cent of normal, calculated from two-stage determinations). The parahemophilic (M. G.) plasma and normal plasma were assumed to contain 0 per cent and 100 per cent Ac-globulin, respectively.

FIG. 10. Prothrombin times of mixtures of normal plasma with deprothrombinated ($BaSO_4$) parahemophilic (M. G.) or normal plasma.

Response of Parahemophilic Defect to Various Agents

Normal Plasma. The clot-promoting effect of normal plasma on parahemophilic blood, shown in Table II, is even more strikingly evident in Figure 12. These observations are in agreement with what Owren found in his original case.

*Dicumarol Plasma.** There is considerable question whether dicumarol causes, besides prothrombinopenia, alterations in other plasma constituents which affect prothrombin conversion. According to Fahey et al.[28] Ac-globulin decreases as much as 50 per cent early in the course of dicumarol therapy, subsequently returning to normal despite continuance of the drug. Quick and Stefanini,[29] as well as we, on the other hand, find no change in L.F. Hurn and colleagues[30] report a "decrease in the conversion of prothrombin to thrombin" in addition to prothrombinopenia, which was attributed to a decrease in "co-thromboplastin."[31] Serum SPCA also declines under the influence of dicumarol.[32]

Our patients presented a unique opportunity

* Obtained from patients with myocardial infarct treated with dicumarol for the prevention of thromboembolic complications.

TABLE V

SPCA ACTIVITY IN PARAHEMOPHILIA

Mixture (ml.)				Prothrombin Time (sec.)	Prothrombic Activity of Mixture†			SPCA Activity (per cent activation‡)	Serum SPCA (units per ml., normal: 14–40)
Normal Plasma	Pathologic* Serum	Saline	Normal $BaSO_4$ Plasma		A Plasma Saline Mixture	B Serum	C Plasma Serum Mixture		
Patient M. G.									
0.05	—	0.05	0.90	49.5	35	—	—	—	—
—	0.1	—	0.90	>75	—	—	—	—	—
—	0.33	—	0.67	41.7	—	14	—	—	—
0.05	0.05	—	0.90	25.6	—	—	120	185	37
Patient A. G.									
0.05	—	0.05	0.90	42.1	48	—	—	—	—
—	0.33	—	0.67	66.7	—	9	—	—	—
0.05	0.05	—	0.90	27.7	—	—	114	118	24

* Sera tested were two hours old.

† Observed activity of the mixtures was corrected for the degree of dilution with the normal $BaSO_4$ plasma.

‡ Calculated from the formula: $\dfrac{C - \dfrac{(2a + b)}{2}}{\dfrac{2a + b}{2}} \times 100$

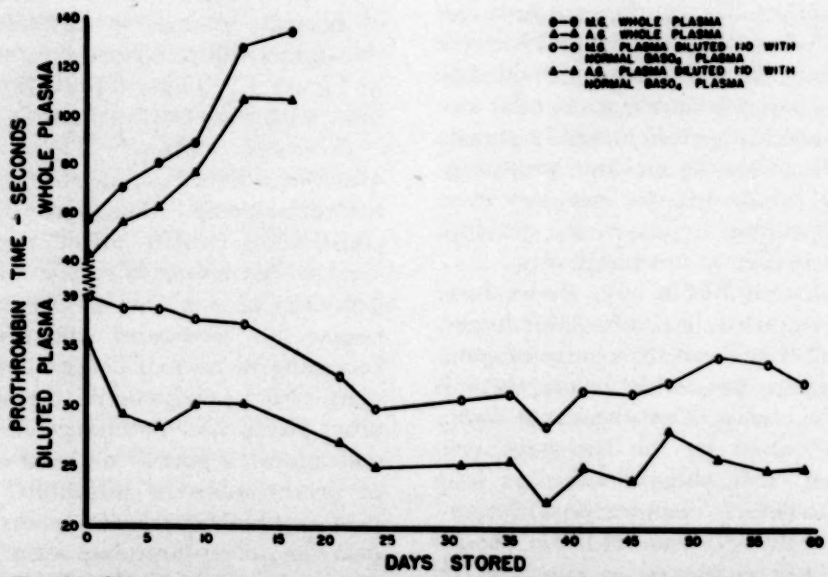

FIG. 11. Changes in one-stage prothrombic activity of oxalated parahemophilic plasma during aging at 4–5°C. Determinations were made at times specified on the whole plasma as well as on a mixture (1:10) of the aging plasma with fresh normal $BaSO_4$ plasma.

of studying this question. It is evident (Table VI) that dicumarol plasma, whole or adsorbed with $BaSO_4$, is normal with regard to its ability to rectify the defect in parahemophilia. These results were paralleled precisely by their rectifying effect, simultaneously observed, on aged normal plasma (data not included here), again supporting the conclusion that the abnormality in parahemophilic plasma and aged normal plasma are identical.

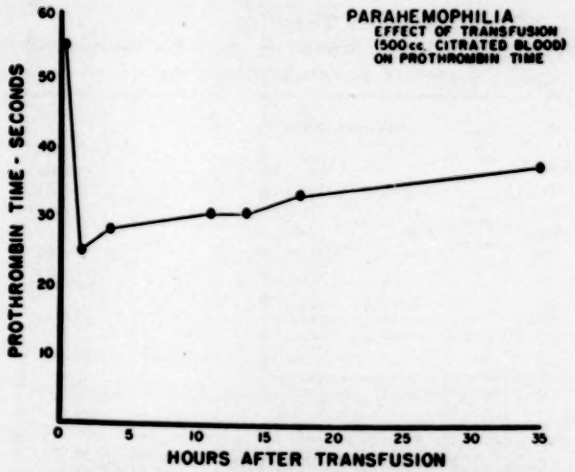

FIG. 12. Effect of transfusion with 500 ml. of fresh citrated blood on the prothrombin time in parahemophiliac (M. G.). It is noteworthy that the clot-promoting effect is practically the same *in vivo* as *in vitro* (compare with Figure 7), assuming the patient's plasma volume to be 2,500 ml. and the plasma volume of the infused blood to be 250 ml. The effect on two-stage prothrombin conversion is seen in Figure 1.

TABLE VI
EFFECT OF DICUMAROL* PLASMA ON PROTHROMBIN TIME OF PARAHEMOPHILIA

Plasma Mixture (Parts)						Prothrombin Time (sec.)
Pathologic Plasma	Normal Plasma	Normal $BaSO_4$ Plasma	Dicumarol Plasma	Dicumarol $BaSO_4$ Plasma	Saline	
M. G.						
4	—	—	—	—	1	84.0
4	1	—	—	—	—	18.1
4	—	1	—	—	—	20.4
4	—	—	1^1†	—	—	21.5
4	—	—	—	1^1	—	20.5
4	—	—	1^2	—	—	20.6
4	—	—	—	1^2	—	21.3

* Dicumarol plasma obtained from patients with myocardial infarct treated with dicumarol for prevention of thromboemolic complications.

† 1^1 Refers to plasma from subject who was on dicumarol for two weeks and had 37 units prothrombin per ml., 9 per cent prothrombin by one-stage method; 1^2 from subject on dicumarol two weeks, plasma prothrombin 46 units, 13.5 per cent.

Phenylindanedione Plasma. Similar reasons led us to investigate the plasma from a patient receiving the prothrombinopenia-inducing drug, phenylindanedione (P.I.D.). No abnormality in Ac-globulin activity was evident (Table VII); the P.I.D. plasma was fully capable of rectifying the defect of parahemophilic plasma as well as that of aged normal plasma. That P.I.D. plasma has a normal amount of Ac-globulin is in accord with the recent observations of Bjerkelund.[33] However, some other clotting disturbance is suggested by the discrepancies between the one- and two-stage prothrombin values. (Table VII.)

TABLE VII
EFFECT OF PHENYLINDANEDIONE (P.I.D.) PLASMA ON CLOTTING DEFECT OF PARAHEMOPHILIA (M. G.) AND AGED NORMAL PLASMA

Plasma Mixture (Parts)					Prothrombin Time (sec.)
Pathologic Plasma	Normal $BaSO_4$ Plasma	Phenindane* $BaSO_4$ Plasma	Aged Normal Plasma	Saline	
4	—	—	—	1	66.4
4	1	—	—	—	24.4
4	0.1	—	—	0.9	34.2
4	—	1	—	—	24.4
4	—	0.1	—	0.9	35.4
—	—	—	4	1	76.2
—	1	—	4	—	19.7
—	0.1	—	4	0.9	34.2
—	—	1	4	—	20.9
—	—	0.1	4	0.9	35.4

* Patient on phenylindanedione for four months for prevention of recurrent pulmonary emboli. His plasma prothrombin was 80 units per ml. by the two-stage method, 10 per cent by the one-stage procedure. It should be noted that there is considerable discrepancy between these values, suggesting some other clotting defect, not Ac-globulin deficiency, in P.I.D. plasma besides prothrombinopenia. Such discrepancies, repeatedly observed, are in process of further investigation.

SPCA Deficient Plasma. The availability of plasma from the patient with congenital SPCA deficiency[25] provided an unusual opportunity of observing whether it could correct the abnormality of parahemophilia. As was to be expected from the earlier demonstration that SPCA deficient blood contained normal amounts of Ac-globulin, the addition of the plasma to parahemophilic blood rectified the defect of the latter. (Table VIII.) The distinction be-

tween SPCA and Ac-globulin is thus further corroborated.

Serum or SPCA. SPCA can accelerate prothrombin conversion in normal, hemophilic, thrombocytopenic, hypoprothrombinemic or heparinized blood but it is relatively inert on aged plasma in which Ac-globulin has largely deteriorated.[14,32,34] Accordingly, it has been concluded that SPCA cannot substantially influence thrombin formation when Ac-globulin is absent. The impotence of purified SPCA* (or serum) on the defect in parahemophilia confirms this. The addition of SPCA or serum to whole parahemophilic plasma failed to lower the prothrombin time. (Table IX.) However, when mixed with the pathologic plasma to which $BaSO_4$ normal plasma was added as a source of Ac-globulin, SPCA as well as serum lowered the prothrombin time markedly.

TABLE VIII
CORRECTIVE EFFECT OF SPCA DEFICIENT PLASMA ON CLOTTING DEFECT OF PARAHEMOPHILIC (M. G.) PLASMA

Plasma Mixture (Parts)				Prothrombin Time (sec.)
Normal Plasma	Normal $BaSO_4$ Plasma	Parahemophilic Plasma	SPCA Deficient Plasma*	
1	—	—	—	14
—	1	—	—	>360
—	—	1	—	54
—	—	—	1	93
1	1	—	—	17
1	—	1	—	17
—	1	1	—	22
—	—	1	1	22
—	1	—	1	76
1	—	—	1	16

* Plasma from patient with congenital SPCA deficiency.[35]

Effect of Vitamin K_1. The importance of excluding vitamin K deficiency as a cause of an elevated prothrombin time in patients with "idiopathic hypoprothrombinemia" has been emphasized by Ley and colleagues.[35] These authors point out that massive doses of vitamin K_1 may occasionally restore the prothrombin time to normal when large doses of water soluble derivatives fail. Although M. G. had shown no improvement following protracted treatment with water soluble synthetic vitamin K, it was deemed advisable to try a massive dose of vitamin K_1. Accordingly, 1.0 gm. of this material was administered intravenously. No change was found twenty-four hours later either in the plasma prothrombin or Ac-globulin concentration.

* Prepared by methods previously described.[14]

TABLE IX
EFFECT OF NORMAL SERUM OR SPCA ON PROTHROMBIN TIME IN PARAHEMOPHILIA (M. G.)

Mixture (Parts)							Prothrombin Time (sec.)
Patient's Plasma	Patient's Serum	Normal Plasma	Normal Serum	Normal SPCA* Preparation	Saline	Normal $BaSO_4$ Plasma	
1	—	—	—	—	—	—	67.2
1	—	—	—	—	—	9	37.7
1	—	—	—	—	1	—	86
1	—	1	—	—	—	—	13.6
4	—	—	1†	—	—	—	52.6
1	—	—	1†	—	—	—	48.2
1	—	—	1†	—	—	18	27.8
1	—	—	—	—	1	18	46.2
1	1	—	—	—	—	18	35.1
1	1	—	—	—	—	—	14.8
1	—	—	—	1	—	—	64.7
1	—	—	—	1	—	18	36.6, 24.8‡

* Solution containing 1 mg. protein, 38 units SPCA, per ml.

† This serum was 24 hours old; it was selected instead of one hour old serum to make sure that only minimal amounts of Ac-globulin were present. All other sera were one hour old. All normal sera contained less than 6 units prothrombin per ml.

‡ The 24.8 second prothrombin time was obtained three minutes after the mixture was prepared, contrasted with the 36.6 second time obtained immediately after the mixture was prepared. This again demonstrates the latent period of interaction of SPCA with a plasma constituent as described in other reports.[14]

Effect of Blood Transfusion. The clot-promoting effect in parahemophilia of normal blood or plasma was clearly shown in Owren's original case. This was studied exhaustively in one of our subjects also. Within two hours 500 ml. of fresh citrated blood were infused into M. G. and observations were made on the bleeding time, clotting time, prothrombin time, Ac-globulin activity and prothrombin consumption. The prothrombin and Ac-globulin activity of the infused blood, determined before infusion, were normal. Its plasma, moreover, was demonstrated to have a rectifying effect on the patient's blood *in vitro*.

Transfusion induced a prompt and fairly well sustained drop in the prothrombin time. (Figs. 12, 13.) Improvement in prothrombin conversion as well as prothrombin consumption was also evident. (Figs. 1, 5.) Nevertheless, although two stage prothrombin conversion was better after transfusion, it was still considerably

subnormal, in harmony with the one-stage results.

Correction of this defect was, furthermore, associated with restoration of the bleeding and clotting times toward normal. (Fig. 5.) On this basis it was believed that extraction of two carious molar teeth could be performed safely if fresh blood was used immediately before and after the procedure. Accordingly, on July 17th the patient received 1,000 ml. of fresh citrated blood (Fig. 13), again with improvement of the clotting defect and bleeding time. The teeth were extracted on July 18th under local anesthesia and a solution of topical thrombin® with an oxycel® pack was applied locally. This was followed with infusion of 200 ml. of freshly processed frozen citrated plasma. No undue bleeding occurred. As a precautionary measure, and because it was believed that the patient's anemia could also be benefited, 1,000 ml. of three day old blood were administered on July 19th. The hemoglobin rose to 10 gm. per cent and convalescence was uneventful. It is noteworthy that the three day old blood appeared to be less effective than fresh blood in correcting the clotting abnormality.

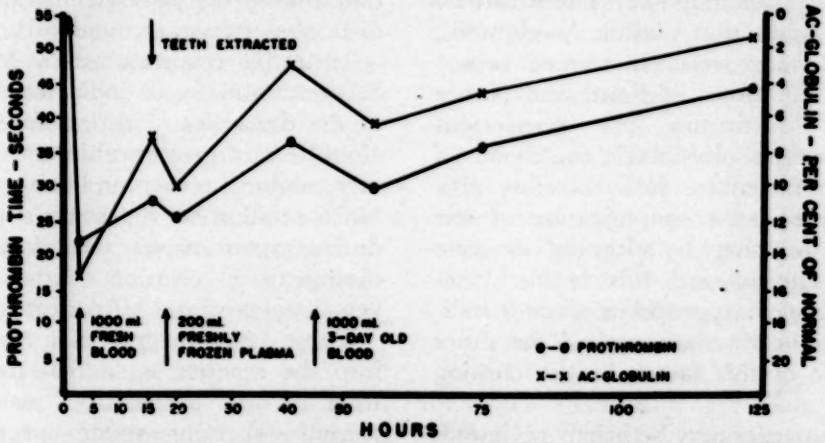

FIG. 13. Prothrombin time and Ac-globulin activity in response to transfusions with fresh blood, freshly processed frozen plasma, or three day old blood. Toward the end of the first 1,000 cc. transfusion, the bleeding time had dropped to seven minutes.

Additional Miscellaneous Studies. That the coagulation defect was the same in all three subjects is indicated by the fact that a mixture comprising equal parts of the three plasmas showed the same abnormalities as the individual plasmas alone.

In view of the elevated bleeding time of the three subjects, their nail bed capillaries were also studied by direct microscopy. No abnormality was detected, such as is found in pseudohemophilia.[36]

To exclude the possibility of hemophilia or hemophilia-like disease the antihemophilic globulin activity of the parahemophilic plasma was measured by observing its rectifying effect on the retarded prothrombin conversion and elevated clotting time of known hemophilic blood. The clot-promoting effect was found normal.

OBSERVATIONS

It is now apparent that parahemophilia is due to a distinct clotting derangement which simulates hypoprothrombinemia clinically and in many laboratory respects despite the fact that the prothrombin concentration may be normal or at least not sufficiently reduced to account for the hemorrhagic phenomena. The disorder may be congenital or acquired, having been observed also in liver disease,[7,37] scarlet fever[38] and leukemia.[39]

Our three patients make a total of eight recorded cases of the congenital type, which include the original one of Owren, one reported by Frank et al.[40] and three by de Vries and colleagues.[11] The disease appears to be frequently associated with other congenital defects. There is as yet no clear-cut evidence that it is also hereditary. The mild hypoprothrombinemia of our subjects, also observed in other cases,[11] is of interest particularly since it failed to respond (in the one case tried) to a massive dose of vitamin K_1. This suggests that, in some instances,

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congenital parahemophilia may be associated with an additional coagulation defect, namely, hypoprothrombinemia, which is resistant to vitamin K_1.[41]

Certain definitive conclusions are now warranted concerning the entity involved in the pathogenesis of parahemophilia. There can no longer be any doubt that plasma Ac-globulin, L.F., factor V (more recently termed proaccelerin[10]), and the factor of Fantl and Nance are identical, constituting one component essential for the rapid physiologic conversion of prothrombin to thrombin. It is therefore now opportune to propose a simplification of our concepts and terminology by adopting one term to designate henceforward this entity; "Ac-globulin" seems most appropriate since it indicates more specifically than any of the other terms* the role of this factor in the clotting sequence.

Its known properties may be briefly reviewed: (1) It is essential for the physiologic conversion of prothrombin to thrombin; (2) its concentration is much higher in bovine, dog and rabbit plasma than in man; (3) it is relatively labile, deteriorating more rapidly in the absence of calcium, and it is more labile in oxalated than in citrated plasma; (4) it is extremely labile in the presence of thrombin; (5) it is not adsorbed by $BaSO_4$, $BaCO_3$, $Ca_3(PO_4)_2$, is slightly adsorbed by $Al(OH)_3$, and partly by $Mg(OH)_2$ and Seitz filters; (6) the principle in plasma (*plasma* Ac-globulin) is converted under the influence of thrombin to a more active form, *serum* Ac-globulin (factor VI, accelerin), which is far more labile; (7) it rapidly disappears from clotting human and canine blood, the sera becoming relatively devoid of it within an hour after coagulation.[28,19] Bovine serum, however, retains large amounts; (8) Ac-globulin precipitates from plasma with the globulins at a pH of approximately 5.3 and is found preponderently in fraction II + III of plasma.[43]

For further details regarding Ac-globulin the reader is referred to the outstanding contributions of Ware and Seegers,[4] and Owren.[44] Much remains to be done on further purification and characterization of the substance in its pure form.

Unless adequate amounts of Ac-globulin are available prothrombin conversion is retarded. This is reflected in the elevated clotting time, slow prothrombin consumption during coagulation and by the poor yield of thrombin (by the orthodox two-stage method). Study of the quantitative relationships by McClaughry and Seegers[45] and by us indicates that with regard to the dynamics of thrombin elaboration, Ac-globulin and prothrombin act in a complementary manner: reduction in either retards thrombin formation to the same degree. Moreover, during spontaneous coagulation Ac-globulin disappears in relation to the amount and/or velocity of prothrombin conversion to thrombin.[19]

These facts suggest that Ac-globulin enters into the reaction as a real participant rather than as an "accelerator" acting outside the thrombin-forming system proper. This view, however, is now open to doubt since despite negligible Ac-globulin activity, prothrombin conversion during coagulation, albeit retarded, proceeds virtually to completion provided sufficient time elapses. This is in agreement with the finding of de Vries et al.[11] Moreover, prothrombin conversion is also complete upon recalcification of aged plasma or commercial and American Red Cross dried plasma, all of which are practically devoid of Ac-globulin activity.[46] It is unlikely, therefore, that Ac-globulin enters the reaction stoichiometrically, an interpretation which is not in agreement with that of Quick and Stefanini.[47]

Some mechanism other than chemical utilization of Ac-globulin must therefore be invoked to explain the disappearance of Ac-globulin during coagulation. It can not be related directly to the conversion of fibrinogen to fibrin since Ac-globulin disappears at a normal rate from freshly shed incoagulable afibrinogenemic blood.[19] Most likely Ac-globulin is destroyed by the thrombin which is evolved[48] or it spontaneously deteriorates rapidly as a consequence of its greater lability after it is converted to the serum Ac-globulin form by thrombin.

The complete prothrombin conversion in the spontaneously clotting parahemophilic blood is somewhat at variance with the negligible conversion and poor thrombin yields obtained in the isolated two-stage system. This may be due in part to the fact that not as much time was allotted in the two-stage system as in the shed

* Recently Stefanini[43] proposed the term "plasma prothrombin conversion factor" (PPCF). In our opinion it is less satisfactory since it adds a new term to an already encumbered literature and at the same time does not distinguish Ac-globulin from other plasma factors which function in prothrombin conversion (e.g., SPCA precursor).

blood (two hours) to ascertain just how far prothrombin conversion could go. Moreover, in the isolated system, prothrombin destruction may occur when prothrombin conversion is very slow, due either to the small amount of thrombin evolved[40] or via other non-specific reactions.[50] In intact blood or plasma, on the other hand, evolved thrombin cannot attain a substantial and persistent titer due to the action of antithrombin.

The question arises as to the best method of measuring Ac-globulin. Parahemophilic plasma, relatively devoid of it, is indistinguishable from aged normal plasma. The elevated prothrombin time of the latter is extremely sensitive to additions of Ac-globulin. This provides a simple and precise means of measuring this factor,[19] a technic far simpler than that involving the two-stage procedure with purified prothrombin.[4]

Certain conclusive statements can now be made regarding the relationship between Ac-globulin and SPCA or its precursor. That they are distinctly different can no longer be disputed: in parahemophilia, SPCA elaboration was normal despite negligible Ac-globulin activity. The same is also found upon recalcification of aged plasma or old lyophilized plasma, both of which are essentially devoid of Ac-globulin. Furthermore, plasma from an SPCA deficient patient corrects the abnormality in parahemophilia. It may also be concluded that SPCA formation during coagulation is apparently independent of and does not require substantial concentrations of Ac-globulin.

It is thus evident that plasma Ac-globulin is not the precursor of SPCA, a view not in accord with Stefanini's.[51] A certain note of caution is, however, indicated. An Ac-globulin activity below 5 per cent of normal does not necessarily indicate that the actual plasma concentration of Ac-globulin protein is that low. Admittedly, deductions concerning absolute quantitative values based upon relative physiologic activities may be fallacious. Also, it is possible that the amount of Ac-globulin required for SPCA formation (on the theory that Ac-globulin is its precursor) may be far less than that needed for the Ac-globulin activity which is concerned with prothrombin conversion. Clearly, a definitive answer must await isolation and characterization of the moieties in pure form. Meanwhile these possibilities are considered most unlikely.

Our finding of a normal SPCA mechanism in parahemophilia is somewhat at variance with the observation of de Vries et al.[11] who found very low SPCA elaboration, even after the prothrombin had been consumed completely. Nevertheless, these investigators found that SPCA formation became normal following the addition of thromboplastin to the freshly shed parahemophilic blood, indicating that the SPCA mechanism was not fundamentally deranged or that at least its precursor in plasma must have been present despite the relative absence of Ac-globulin.

It is now evident that both Ac-globulin and SPCA, or its precursor, are required for the rapid physiologic conversion of prothrombin to thrombin by thromboplastin and calcium. Deficiency of either results in retarded thrombin formation, associated with defective hemostasis, simulating hypoprothrombinemia. Neither can replace the other: SPCA supplements cannot lower the elevated prothrombin time of parahemophilic plasma. Conversely, inadequate amounts of SPCA or its progenitor result in delay in the early activation of prothrombin by thromboplastin, a defect which can be corrected only by restoration of the missing factor.

In the light of this it would now seem appropriate to reclassify the hypoprothrombinemias as follows:

GENERAL HYPOPROTHROMBINEMIAS

I. True hypoprothrombinemia
   - A. Congenital, ? familial
   - B. Acquired
     1. Idiopathic
     2. Liver disease
     3. Vitamin K deficiency
     4. Drug-induced

II. Pseudohypoprothrombinemia
   - A. Ac-globulin deficiency (parahemophilia)
     1. Congenital, ? hereditary
     2. Acquired: liver disease, purpura fulminans
   - B. SPCA deficiency
     1. Congenital
     2. Acquired: ? dicumarol poisoning.

The beneficial effect in parahemophilia of transfusion with normal blood or plasma is of profound clinical import. To have reduced the prothrombin time from 56 seconds to 26 (by 500 ml. of fresh blood, Fig. 13), or from 55 seconds to 22 (by 1,000 ml., Fig. 14) is tantamount to increasing prothrombic activity from approximately 3 to 25 per cent of normal, a level

which is consistent with effective hemostasis if other hemostatic mechanisms are normal. The beneficial effect is also attested to by the normalization of the clotting and bleeding times, and of the prothrombin consumption. Our experience also demonstrates that elective surgery may be performed safely if blood and plasma are liberally used. It should be emphasized that they must be relatively fresh because Ac-globulin deteriorates fairly rapidly under ordinary conditions of storage.

Also important from the standpoint of management is the rate with which Ac-globulin disappears from parahemophilic blood following transfusion. In agreement with Owren's observations, the clot-promoting effect of transfusion was still detectable for more than twenty-four hours. This is in distinct contrast to the relatively rapid rate with which prothrombin disappears from the circulation in congenital hypoprothrombinemia[24] or SPCA in congenital SPCA deficiency.[52]

The elevated clotting and prothrombin times need no additional comment except to state that when prothrombic activity is markedly reduced, distinct slowing of over-all coagulation supervenes. Retarded clotting is also seen in less severe hypoprothrombinemic states when special technics are employed, such as the use of siliconized clotting time tubes.

Of greater interest, however, is the markedly elevated bleeding times of our subjects. This abnormality is generally attributed to capillary abnormalities such as have been described in thrombocytopenia or pseudohemophilia[35] but which were not evident in our cases. It is hard to imagine how Ac-globulin can be directly related to a disturbance in the response of capillaries to trauma. It is also difficult to attribute the elevated bleeding time solely to retarded thrombin evolution and the attendant delay in platelet agglutination, since in hemophilia, a condition notoriously associated with comparably high clotting times, with poor prothrombin conversion and with delayed platelet agglutination, the bleeding time is normal. This paradox may plausibly be explained by postulating that tissue thromboplastin plays an important role normally in controlling hemorrhage following a lancet stab. In parahemophilia, thromboplastin even in excess cannot induce adequate thrombin formation because of the blocked prothrombin conversion whereas in hemophilia, tissue thromboplastin can accelerate thrombin formation, the prothrombin conversion mechanism being normal.

It is of interest that of the three parahemophiliacs, M. G. with the highest prothrombin time was the only one who experienced frank hemorrhagic phenomena. This correlation is thoroughly consistent with clinical and laboratory experience regarding the relationship between pathologic bleeding and the degree of true hypoprothrombinemia, drug-induced or acquired from disease.

SUMMARY

A clinicolaboratory study is presented of parahemophilia in three siblings, apparently congenital in origin. On the basis of the data it can now be concluded that the disorder, constituting a form of pseudohypoprothrombinemia, is referable to deficiency of a non-prothrombin plasma constituent which is essential for the rapid physiologic conversion of prothrombin to thrombin by thromboplastin and calcium. This substance, for which the term Ac-globulin (Ware and Seegers) is considered most appropriate for simplification of nomenclature, is identical with labile factor (Quick), factor V or proaccelerin (Owren), and the factor of Fantl and Nance. The substance is present in fresh normal whole blood or plasma, which provides the basis for effective clinical management of parahemophilia. Also, it is unequivocally distinct from SPCA and its precursor, deficiency of which results in another form of pseudohypoprothrombinemia.

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The Limit of Hemoglobin Synthesis in Hereditary Hemolytic Anemia*

Its Relation to the Excretion of Bile Pigment

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If the bone marrow possessed an unlimited capacity for erythropoiesis, no anemia would develop in hemolytic disease. As fast as red cells were destroyed they would be replaced. Even without such an ability the marrow, as it actually exists, can compensate for a considerable amount of abnormal blood destruction. Anemia results only when the hemolytic process overtaxes the regenerative capacity. The limit of the human bone marrow's ability to produce red cells in response to increased demand has never been established.

In chronic hemolytic disease, as exemplified by the hereditary hemolytic anemias, the regenerative effort of the bone marrow appears to be rather constant. Unless some sort of crisis intervenes the hemoglobin level of such patients, with or without anemia, tends to remain fairly static month after month. Hemolysis and erythropoiesis are in equilibrium, even as they are in normal people, but the rate of exchange is faster than normal. The existence of anemia in a patient with chronic, stabilized hemolytic disease suggests that his bone marrow is working at or very near its capacity but despite the effort is unable to produce enough hemoglobin to maintain a normal level in the circulation. In order to ascertain the limits of hemoglobin and red cell synthesis during the sustained heavy demand of such a disease, we have examined the problem in two patients with hereditary hemolytic anemia.

MATERIAL AND METHODS

The subjects of this investigation were young men, both members of the United States Armed Forces. The first had hereditary spherocytosis. He was admitted to a military hospital for an acute respiratory infection and the presence of splenomegaly drew attention to his underlying disease. The diagnosis was confirmed by a family study. The anemia was later cured by splenectomy. The second patient, together with his family, has been the subject of a previous report.[3] Although the hemolytic anemia was hereditary, it was different from hereditary spherocytosis for there were no spherocytes, and splenectomy proved to be of no value. The disease was also different from other known forms of hereditary and familial anemia. The patient was originally admitted to the hospital with an acute arthritis which had subsided long before the present study was begun.

The bone marrow of both patients, aspirated on several occasions from various sites, showed marked erythroid hyperplasia. Over a period of observation of many consecutive months, the degree of anemia and jaundice of these patients was found to be quite constant. The hematocrit did not vary ± 2 from the values shown in Table 1. The plasma bilirubin ranged between 1.8 and 4.0 mg. per 100 cc. Thus both patients had a chronic, moderate, well stabilized hemolytic anemia with generalized hyperplasia and hyperactivity of the bone marrow. Both were healthy except for the blood dyscrasia and both were well nourished on a good hospital diet.

The procedure of the investigation was the same for both patients. The red cell volume was established by the method of Mollison.[16] Both were of blood group A. From each patient 500 cc. of blood were withdrawn into ACD mixture. Then each was immediately given a rapid transfusion of 500 cc. of normal, fresh group O blood. In each patient's circulating blood the number of red cells unagglutinable by

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anti-A serum was determined just before the transfusion and thirty minutes after. The increase of unagglutinable red cells represented the transfused cells. The total volume of red cells in the patient's circulation prior to the bleeding and transfusion was calculated by this formula:

$$\text{Vol RBC} = \frac{VP}{I} + (V - v)$$

Where P = patient's red cell count in millions, I = increase of the unagglutinable RBC count

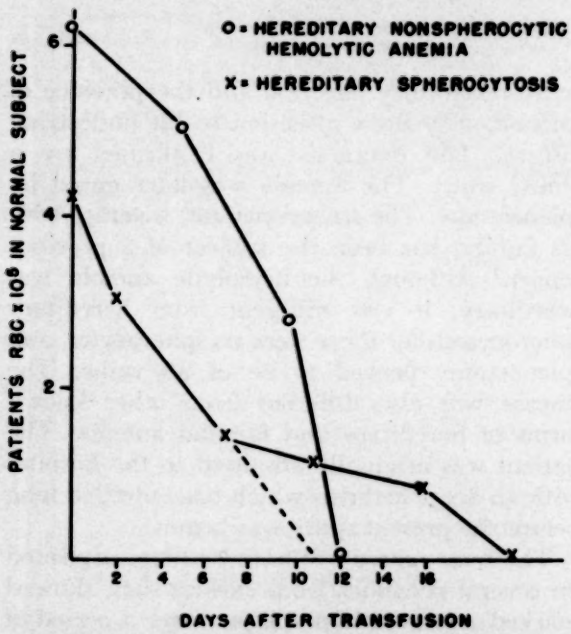


FIG. 1. The average survival time of red cells of patients with hereditary hemolytic disease after transfusion into normal recipients. The curve of decay of the non-spherocytic cells approximated a straight line, and their average life span was taken to be twelve days. The average life of the spherocytic cells was estimated to be eleven days. This was found by extending to the base line the initial rapidly falling portion of the curve of decay. Mathematical justification of this procedure has been presented by Dornhorst.[7]

in millions, V = volume of RBC transfused into the patient and v = volume of RBC donated by the patient. The values for V and v were calculated from hematocrits of blood taken from the transfusion bottles.

The blood that was withdrawn from each patient was immediately transfused into a healthy recipient of blood group AB. Survival of the transfused cells was determined by Young's modification of the Ashby method.[31] The average life span of the transfused cells was established by procedures described by Dornhorst[7] and demonstrated in Figure 1.

The daily production of red cells (in cubic centimeters) was determined by dividing the total red cell volume (in cubic centimeters) by the red cell survival time (in days). Because the hemolytic-erythropoietic system was in equilibrium, this figure also represents the daily destruction of red cells. The daily production and destruction of hemoglobin was estimated to be one-third the mass of the red cells, the cells being normochromic.

Fecal urobilinogen was determined by Sparkman's method.[26] Each day's specimen was analyzed separately and the results of four or more consecutive days were averaged.

RESULTS

The results of these investigations are summarized in Table I and in Figures 1, 2 and 3.

The patient with hereditary spherocytosis donated red cells whose average life span in a normal recipient was eleven days. The figure agrees well with the findings of others who have determined the survival of the abnormal red cells of this disease.[4,15,20] The average suvival of the red cells from the patient with non-spherocytic hemolytic anemia was twelve days. Mollison has recently determined the survival of the red cells from such a patient and found it to be fifteen days.[19]

The normal red cells transfused into both patients survived normally. The rate of disappearance in each case indicated a life span of more than 100 days. The total red cell volume of both patients was found to be diminished by about 25 per cent, as compared with the normal. When the total red cell volume was divided by the average life span of the red cells, to learn the proportion replaced each day, it was found that the rate of erythropoiesis was approximately seven times greater than the normal. (Table I.)

The average daily excretion of urobilinogen in the patient with hereditary spherocytosis was 760 mg. for a period of five days. The fecal urobilinogen of the patient with non-spherocytic hemolytic anemia was determined each day for fifty-one consecutive days (Fig. 2) and on another occasion for fourteen days. (Fig. 3.) On the latter occasion the daily urinary urobilinogen was also determined. When the values were averaged in four-day aggregates, a "cyclical" pattern of excretion was revealed, the cycles extending over about twenty-five days. The amplitude of variation was great, amount-

ing to a difference of some 1,500 mg. per day between the highest and the lowest averages. The descending slope of each curve was steeper than the rising slope. The variations were unrelated to the patient's bowel habits. There was no consistent relationship between the weight of the feces and the amount of urobilinogen. For example, the four days' output of urobilinogen, averaged to obtain the figure of 405 mg. on the eight day in Figure 2, was contained in four stools that averaged 210 gm. in weight. On the twenty-fourth day in Figure 2 the urobilinogen output is shown to be 2,050 mg. The average weight of those four stools was 181 gm. The

TABLE I

THE PRODUCTION OF RED BLOOD CELLS AND HEMOGLOBIN BY PATIENTS WITH CHRONIC HEMOLYTIC ANEMIA*

	Body Weight (kg.)	RBC (millions per cu. mm.)	Hematocrit	Hb (gm./100 cc.)	RBC Donated (cc.)	RBC Received (cc.)	Increase of Inagglutinable RBC after Transfusion (millions per cu. mm.)	Volume of RBC		Life Span of RBC (days)	Daily Production			Fecal Urobilinogen (mg./day)	
								Total (cc.)	(cc./kg.)		RBC (cc.)	Hb (gm.)	Hb (gm./kg.)	Anticipated	Observed
1. Hereditary spherocytosis	62	4.1	37	13.0	185	225	0.7	1,325	21.5	11	120	40	0.64	1,400	760
2. Non-spherocytic anemia	70	3.94	35	11.5	175	225	0.53	1,630	23	12	136	45	0.63	1,575	1,300
3. Normal	70	....	..	....	...	...	....	2,250	32	120	18.8	6.25	0.09	220	50–250

* The normal is based upon the best available figures for red cell mass[27] and red cell survival time.[1]

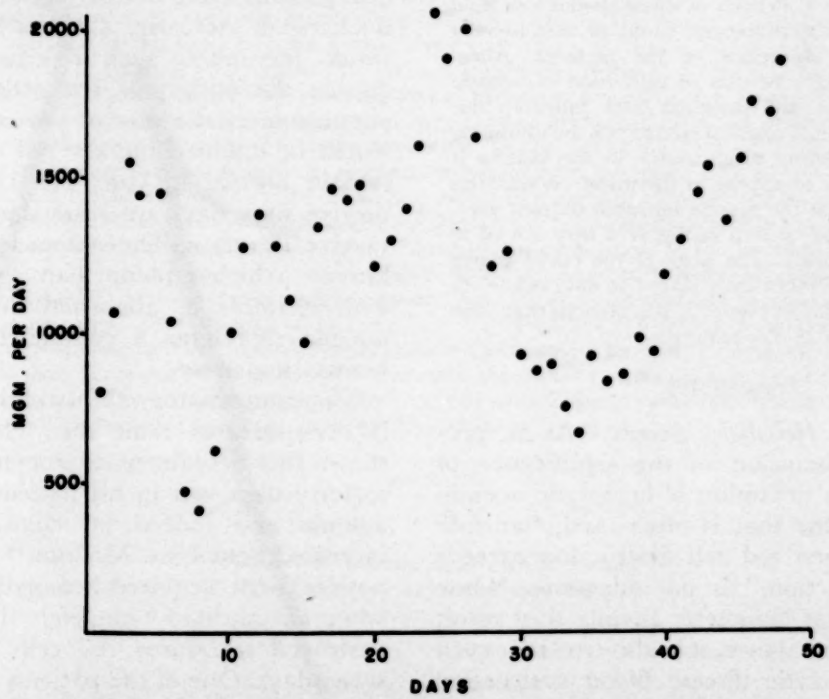


FIG. 2. An apparently cyclic pattern of fecal urobilinogen excretion in a patient with hereditary non-spherocytic hemolytic anemia. Each point represents a four-day average; the first point is the average of days 1–4, the second of days 2–5, etc. Although only 48 points are shown, the data cover a period of fifty-one days. During this time the patient's hemoglobin and plasma bilirubin remained at a constant level, and he remained in good health. The urobilinogen excretion appears to vary according to a 25-day cycle.

average daily excretion of urobilinogen for the twenty-five-day cycle was 1,250 to 1,350 mg. The urinary urobilinogen was found to vary inversely with the changes in fecal urobilinogen. (Fig. 3.)

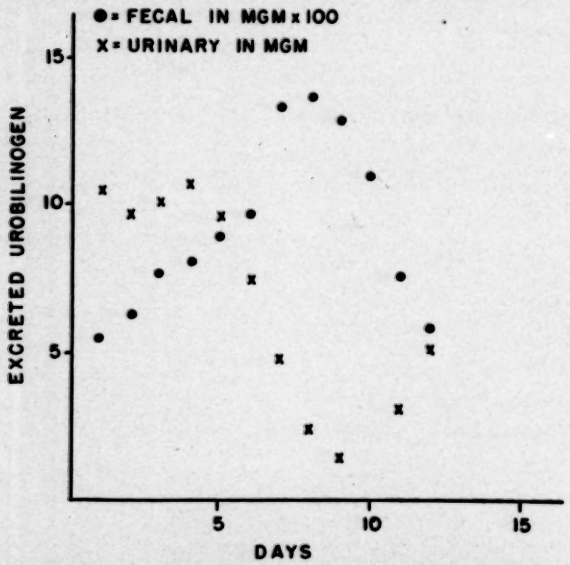


FIG. 3. An apparently inverse relation between fecal and urinary urobilinogen excretion. The patient is the same as in Figure 3. Periods of simultaneous low fecal and high urinary urobilinogen could be due to very active intestinal absorption of the pigment. Alternatively, they might be due to inhibition of hepatic excretory function. By damming back bilirubin this would cause the fecal urobilinogen to fall; by damming back the recirculating urobilinogen in the plasma it would cause more to appear in the urine. Against this stands the fact that the plasma bilirubin did not vary. The 25-day "cycle" shown in Figure 4 may not be as regular as it appears. The peak of the curve shown above fell on November 25th, sixty-five days before the peak of the 29th day in Figure 3. It is obvious that these peaks do not fit a 25-day pattern.

COMMENTS

Definition of Hemolytic Anemia. As a preliminary to discussion of the significance of these findings a definition of hemolytic anemia is necessary. One that is often used, "anemia that results when red cell destruction exceeds red cell production," is not adequate. While it is obvious that hemolytic anemia does result from such an imbalance, it is also true that even in severe hemolytic disease blood destruction cannot exceed blood production for any considerable length of time. Destruction can exceed production only at the expense of the red cells in the circulation, and a falling red cell count is evidence of this unbalanced situation. It occurs during hemolytic crises, but in chronic hemolytic disease the balance between red cell production and destruction is usually in fairly good equilibrium. A constant level of hemoglobin, however low, indicates that production of hemoglobin equals destruction.

Implicit in the argument is the conclusion that when the production of red cells is constant the number of red cells in the circulation depends upon their life span. In the circulating blood of a normal man of 70 kg. the volume of red blood cells is approximately 2,250 cc.,[27] equivalent to 750 gm. of hemoglobin. It is well established that the average survival time of normal red cells in a normal circulation is about 120 days.[1] Each day $\frac{1}{120}$ of the red cell mass is destroyed and replaced. This exchange involves approximately 18.18 cc. of red cells or 6.25 gm. of hemoglobin. It is a function of the bone marrow to produce red cells in sufficient quantities to maintain a normal volume of hemoglobin in the circulation. If the survival time of the red cells were reduced by half (sixty days) without a compensating increase of erythropoiesis, the circulating red cell mass would be reduced by half, although the number of red cells produced and destroyed each day remained unchanged. Actually, a normal bone marrow would respond to such a demand and would double the output of red cells. Under these circumstances the rate of red cell replacement would be doubled but the red cell mass would remain normal in size. Ten cc. of cells that survive sixty days are equivalent to 5 cc. that survive 120 days. The system is in equilibrium but on a higher plane than normal. (Fig. 4.) This example of abnormal hemolysis *without* anemia represents a completely compensated hemolytic disease.

The compensatory ability of the bone marrow is even greater than this. Hedenstedt[10] has shown that hereditary elliptocytes survive thirty to forty days, yet in his patients there was no anemia and indeed no clinical evidence of increased hemolysis. Mollison[18] has described a patient with acquired hemolytic disease who, when anemia had completely disappeared, still destroyed transfused red cells within twenty-seven days. One of the patients with hereditary spherocytosis studied by Dacie and Mollison[4] had red cells with an average life span of twenty-five days. This patient was not anemic.[19] When the red cell survival time is thirty days, the bone marrow in order to prevent anemia must elaborate four times the normal require-

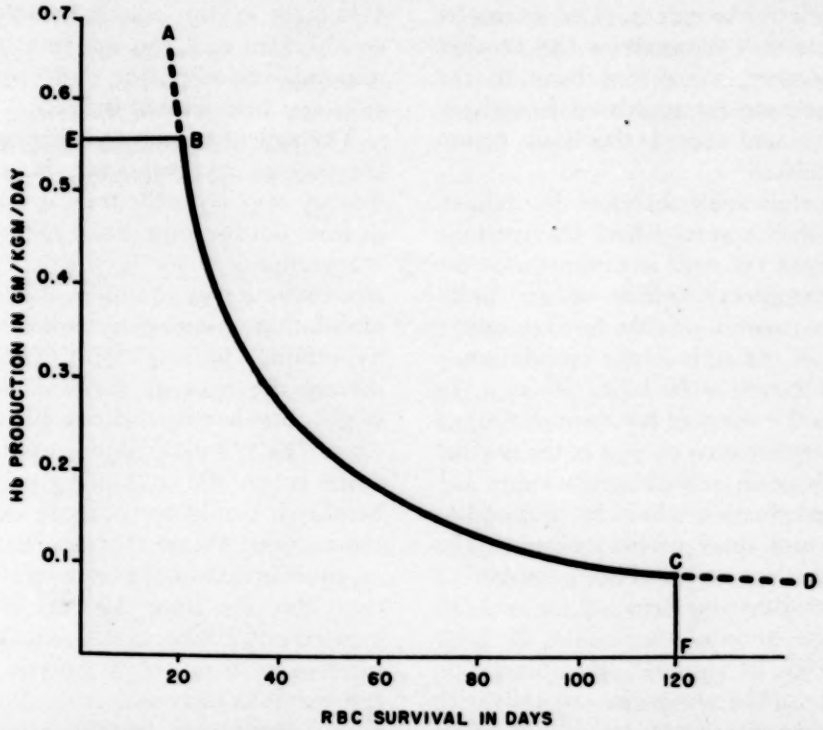


FIG. 4. Pathogenesis of anemia: The normal volume of circulating hemoglobin is approximately 10.75 gm. per kg. of body weight (750 gm. in a 70 kg. man). This mass of hemoglobin is maintained by a balance between erythropoiesis and hemolysis. Since the average survival of normal red cells is 120 days, it follows that the entire mass of hemoglobin in normal people is replaced every 120 days. In hemolytic disease the exchange of new red cells for old is accelerated, but anemia does not result if erythropoiesis is adequate. So far as the mass of hemoglobin is concerned, 60 red cells that survive only 12 days are the equivalent of 6 cells that survive 120 days. This compensatory relation of hemoglobin synthesis and survival is expressed in this figure by the curve AD. When the survival time is shortened, as in hemolytic disease, more hemoglobin must be produced in order to maintain the normal volume of hemoglobin in the circulation. The portion of the curve BC represents the range in which complete compensation is possible. It presents the requirement for hemoglobin as well as the production. The limit of the bone marrow's ability to increase production imposes a physiologic barrier at point B. If the survival of hemoglobin (i. e., red cells) grows even shorter, the requirement continues to mount along the line BA, but production is fixed at BE. The degree of anemia that exists under those circumstances is proportional to the difference between requirement and supply. At the other end of the curve where the hemoglobin survives for a normal 120 days the same principle applies. When the bone marrow fails to provide an adequate replacement, an extension of the life span of the red cells in the direction CD would be a compensating device. But there is another physiologic barrier at this point. Few red cells survive beyond 120 days. When the number produced is less than the requirement, as in aplastic anemia, the need for a compensatory extension of the red cell life span is indicated by CD. Actual production descends along CF and again the anemia is proportional to the difference. The degree of anemia may be formulated thus:

$$A = \frac{S \times 100}{R}$$

Where A = anemia in per cent of normal,
S = actual supply of hemoglobin and
R = the requirement.

Otherwise

$$A = \frac{t \times hb}{T \times Hb} \times 100$$

Where t = observed survival time of hb (in days),
hb = observed production of hb per day,
T = normal survival (120 days) and
Hb = normal production (0.09 gm./kg./day).

ment for red cell replacement. The examples just cited indicate that the marrow can do this. Somewhere, however, there is a limit to the ability to compensate for increased hemolysis. When the requirement exceeds this limit, hemolytic anemia results.

Hemolytic anemia may therefore be defined as an anemia that results when the average survival time of the red cells is so short that the maximum erythropoietic effort of an uninhibited bone marrow is unable to maintain a normal volume of red cells in the circulation.

Pathogenesis of Anemia in Hemolytic Disease. In our two patients the average life span of the red cells was about twelve days or $\frac{1}{10}$ of the normal 120 days. With such red cells ten times the normal rate of production would be required to maintain a normal mass of hemoglobin. The requirement was not met. The presence of anemia indicates that the demand for red cell replacements had exceeded the limit of the bone marrow's capacity to respond. By correlating the survival time of the abnormal red cells with the patient's total red cell volume, it was found that the bone marrow—although hyperplastic, well nourished and stimulated by chronic anemia—was able to produce about seven times the normal amount of red cells and hemoglobin per day. (Table I.) The marrow apparently reached the limit of its ability to compensate when the average red cell survival time was reduced to about fifteen to twenty days. At that rate of hemolysis, six to eight times the normal, the hyperactive marrow of our patients could, theoretically, have maintained a total red cell volume of normal size. They became anemic because the average life span of their erythrocytes was even less.

The degree of anemia that develops under these conditions is proportional to the difference between the actual red cell production and the amount of red cells required to keep the total volume at a normal level. (Fig. 4.) For example, if an ideal red cell volume in a hypothetic patient was 2,000 cc. and the limit of erythropoietic capacity was 100 cc. of red cells per day, there would be enough red cell production to maintain the red cell volume when the life span of the cells was no less than twenty days ($100 \times 20 = 2{,}000$). If, however, the cell survival were ten days there would be only half enough red cells, and the red cell mass would then be 1,000 cc. ($100 \times 10 = 1{,}000$). With a five-day survival the red cell mass would be 500 cc. When the erythropoietic-hemolytic system is in equilibrium and the marrow is working at its maximal capacity, the daily production of red cells and hemoglobin is fixed.

The rate of hemolysis is necessarily limited by the rate of erythropoiesis. It is not possible to destroy *more* red cells then are being produced, at least not for long. Such spendthrift hemolysis is accomplished only at the expense of a progressive reduction of the mass of hemoglobin in the circulation. During a hemolytic crisis in the hypothetic patient with chronic hemolytic disease the average survival time of red cells might be shortened from fifteen days to five days. The red cell volume would then fall from 1,500 cc. to 500 cc. During this fall the rate of hemolysis would temporarily exceed the rate of destruction. When the new level was reached, an equilibrium would be re-established although each day the same 100 cc. of red cells were created and 100 cc. destroyed. During the period of recovery from the hemolytic crisis, when red cell survival increased from five days to fifteen days, production would temporarily exceed hemolysis. A similar temporary imbalance occurs when the marrow reduces its output as it apparently does during the aregenerative (aplastic) crisis of chronic hemolytic anemia.[20,25] During this crisis the total volume of red cells decreases, not because of a change of the red cell life span but because production has been curtailed. Nevertheless, hemolysis outstrips production until the system achieves equilibrium at the new level. The severity of the anemia would, of course, be proportional to the difference between the requirement for red cell replacement and the supply. (Fig. 4.) During recovery from an aregenerative crisis, production *exceeds* hemolysis until the red cell volume has re-expanded.

These simplified examples serve to illustrate the pathogenesis of anemia in hemolytic disease and to emphasize the fact that the red cell volume in the circulating blood depends upon two factors: the rate of erythropoiesis and the average life span of the red cells.

Limits of Hemoglobin Synthesis in Hemolytic Disease. It is open to question whether a hemoglobin output of 0.60 to 0.65 gm. per kg. of body weight per day represents the limit of erythropoietic capacity of normal adults. An output of this magnitude, as demonstrated in our two patients, is probably fairly representative of most adults with a chronic stabilized hemolytic anemia of moderate intensity. In Table II the

figures for several types of chronic hemolytic anemia are given. It is seen that the rate of hemoglobin production in sickle cell anemia is only a little less than it was in our two patients. In Cooley's anemia, pernicious anemia and paroxysmal nocturnal hemoglobinuria the production of hemoglobin is considerably less, being only three to four times the normal rather than six to eight times. In these latter diseases a *relative* inhibition of bone marrow appears to exist.

TABLE II

PRODUCTION OF HEMOGLOBIN IN VARIOUS CHRONIC HEMOLYTIC DISEASES ASSOCIATED WITH ABNORMAL RED CELLS*

	Hemoglobin (gm./100 cc. of blood)	Life Span of RBC (days)	Production of Hb (gm./kg./day)
Sickle cell anemia[3]			
Case L. B.	9.3	14	0.43
Case B. W.	9.9	13	0.50
Case H. G.	9.8	12	0.53
Pernicious anemia[24]			
Case 1	8.4	20	0.27
Case 2	8.7	25	0.23
Case 3	8.0	25	0.21
Cooley's anemia[11]			
Case 1	5.4	20	0.18
Case 3	9.0	30	0.20
Case 4	7.8	16	0.30
Nocturnal hemoglobinuria[5]	8.0	15	0.35

* This information is taken from several reports of red cell survival. The average life span was determined by the method of Dornhorst. (Fig. 1.) The blood volume is estimated to be 65 cc. per kg. of body weight. The daily production of hemoglobin in grams per kg. of body weight is calculated on the following formula:

$$P = \frac{0.65 \times Hb}{T}$$

Where Hb is the concentration, in grams per 100 cc. of hemoglobin in the circulating blood and T is the average life span of the patient's red cells. The production of hemoglobin in normal adults is about 0.09 gm. per kg. per day. (Table 1.)

In chronic hemolytic disease the bone marrow reacts to compensate for increased blood destruction by producing hemoglobin at four to eight times the normal rate. In most patients and in most of these chronic diseases the rate seems to be about six or seven times the normal. From this it may be concluded that in such cases the bone marrow is able to compensate completely for a hemolytic process in which the average life span of the red cells is reduced to about twenty days or 1/6th of the normal. When the life span becomes shorter than that, anemia results.

Is the bone marrow capable of a further effort should the anemia be more severe?* The animal experiments of Whipple's group[22] indicate that in chronic *moderate* hemorrhage anemia the regeneration of hemoglobin was less than that found in severe anemia but these figures were not corrected for the loss of blood occurring by normal attrition. However, it is possible that the synthesis of hemoglobin in chronic hemolytic anemia is greater than our estimates indicate. The average survival time of these patients' abnormal red cells was determined with transfused blood. The method does not take into account the possibility that the bone marrow may produce a portion of extremely fragile red cells with so short a survival time that they are not represented in the transfused blood. Evidence in support of this conjecture is to be found in the urobilinogen excretion of our patient with non-spherocytic anemia. Periodically the rate of excretion approximated 2,000 mg. per day (Fig. 2) which implies a rate of hemolysis and hematopoiesis of about 0.80 mg. per kg. per day. Further indirect evidence of an extremely fragile component of red cells has been provided by experiments with N^{15}-labelled hemoglobin reported by Shemin and his associates.[13,14] Isotopic hemoglobin was produced when glycine that contained N^{15} was fed to a subject. The isotopic nitrogen was incorporated into the porphyrin of the hemoglobin molecules. When the red cells were destroyed, the subject's feces were found to contain isotopic (N^{15}) urobilinogen derived from the porphyrin. The peak of the excretion of N^{15} urobilinogen occurred about 125 days after the feeding of glycine, indicating the average survival time of the red cells. But there was also a minor peak of urobilinogen excretion which occurred just after the glycine was fed, during the production of N^{15}-labelled red cells. This initial excretion of N^{15} urobilinogen has been widely interpreted to indicate that there are sources of bile pigment other than hemoglobin. London[14] has suggested, as an alternative explanation, that part or all of the early appearing urobilinogen may be derived from extremely short-lived red cells. In normal men and women the early isotopic urobilinogen amounted to about 10 per cent of

* A very high proportion of reticulocytes in severe hemolytic anemia does not necessarily reflect an increase of hemoglobin production beyond that encountered in moderate hemolytic anemia. It probably occurs because the red cells are being released earlier, though in no greater numbers, and because few of the red cells survive beyond the time required for maturation of reticulocytes.

the total daily excretion.* In sickle cell anemia and pernicious anemia, both of which are chronic hemolytic diseases associated with defectively formed red cells, the early isotopic urobilinogen amounted to about 30 per cent of the daily totals. This suggests that a large proportion of the bone marrow's erythropoietic capacity in these diseases is "wasted" in the futile production of abortive, completely nonviable red cells. For example, the extensive phagocytosis of *nucleated* red cells in the bone marrow of pernicious anemia indicates that much of the hemoglobin synthesized in this disease never reaches the peripheral circulation.[6,21] If such were the usual case in other chronic hemolytic anemias, the computed output of 0.6 gm. of hemoglobin per kilo per day would be in error. But, at the same time, it would represent the limit of erythropoietic capacity even though it measured only the output of "useful" hemoglobin.

The possibility that transfused red cells survive differently in a normal circulation than they do in their native habitat cannot be gainsaid. That possibility is a recognized limitation of the Ashby method but the results of the method generally correlate well with results obtained by other more intricate technics.[1]

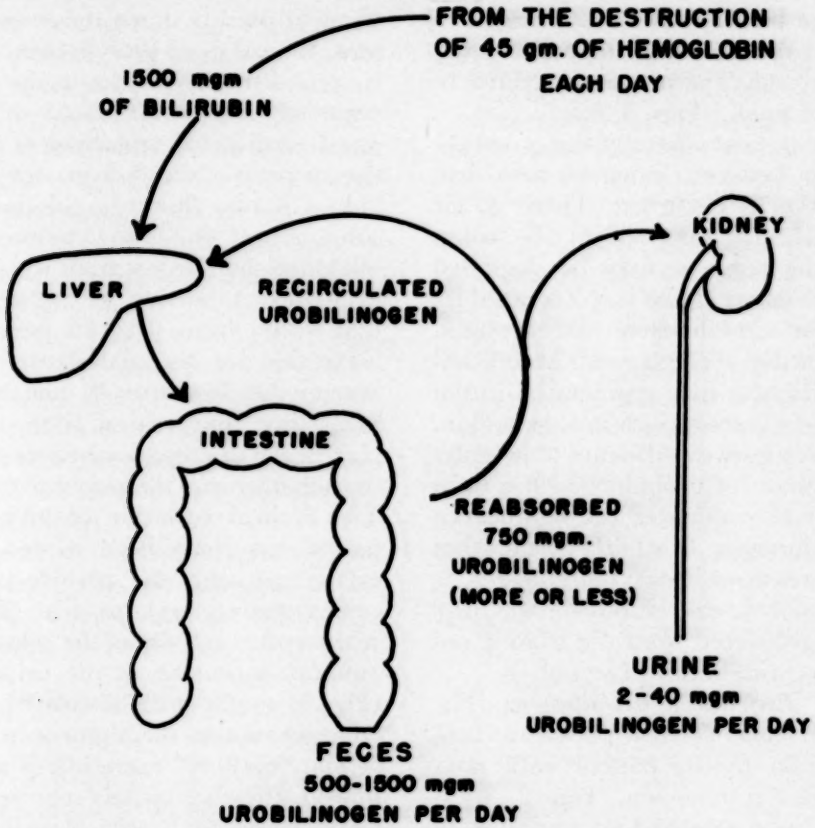
The Relation of Erythropoiesis to the Formation of Bile Pigment. (*Fig. 5.*) The theory now goes that the porphyrin components of the hemoglobin molecule are degraded to bilirubin when the red cell is hemolyzed. The bilirubin is excreted by the liver into the bowel where it is reduced to urobilinogen. Each gram of hemoglobin is believed to be capable of producing about 35 mg. of bilirubin from which, theoretically, a like amount of urobilinogen should be derived.[12] The rate of excretion of bile pigment is therefore a reflection of the rate of hemolysis and, like the rate of hemolysis, is a function of the rate of red cell production and the average life span of the red cells. A normal 70 kg. man who elaborates and destroys 6.25 gm. of hemoglobin each day should produce 220 mg. of urobilinogen. A 70 kg. patient with chronic hemolytic disease whose marrow is working at or near capacity to produce 40 gm. per day of hemoglobin should produce 1,400 mg. per day of bilirubin. Rarely in either case does the actual amount of urobilinogen recovered from the feces equal the amount anticipated. (Table 1.)

The constancy of the plasma bilirubin level indicates that the liver excretes bile pigment at the same rate at which it is formed.* The high level of plasma bilirubin in chronic hemolytic anemia indicates that the excretory function lags behind production by several hours. The excretion of bilirubin moves apace with bilirubin production but does not keep abreast.

The excretion of bile pigment by the liver is believed to represent faithfully the amount of hemoglobin that is destroyed. At approximately normal rates of hemolysis, experiments have shown that 90 to 100 per cent of anticipated bile pigment can actually be recovered from a bile fistula.[9] This bilirubin is changed in the bowel to urobilinogen. Excretion of the latter pigment is rather variable. When collections of normal feces are made over long periods of time, the amount of urobilinogen recovered each day (from the exemplary 70 kg. man) usually falls short of the anticipated 220 mg. The normal range is stated to be 50 to 250 mg. per day for normal adults.[17,28] Miller, Singer and Dameshek[17] correlated fecal urobilinogen excretion with the hemoglobin mass and found emperically in normal healthy subjects that 10 to 20 mg. were excreted for each 100 gm. of circulating hemoglobin. Assuming a normal red cell survival time of 120 days there should have been 29 mg. if all of the bilirubin had been converted to urobilinogen and excreted. The same sort of discrepancy is noted in chronic hemolytic disease. It was calculated above that an adult with chronic hemolytic anemia in a state of equilibrium destroys each day enough hemoglobin to provide about 1,500 mg. of bilirubin. Our second patient showed a fairly good correspondence between the anticipated (1,575 mg.) and the actual (1,300 mg.) excretion of urobilinogen. The patient with hereditary spherocytosis did not. The anticipated urobilinogen excretion was 1,400 mg. per day but the actual excretion was half of that. The low value agrees well with

* If one assumes that the early-appearing 10 per cent of urobilinogen originates, in large part, from the hemoglobin of nonviable red cells, the daily production of hemoglobin in a normal man would be 0.1 mg. per kg. rather than 0.09 as computed in Table 1. In any case the anticipated excretion of urobilinogen would be increased by 10 per cent. In a 70 kg. man it would be 240 mg. per day.

* If the 1,400 mg. of bilirubin formed each day in chronic hemolytic disease were not excreted at all, the pigment would accumulate in the plasma at the rate of 60 mg. per hour, which is enough to raise the concentration of bilirubin in 3,000 cc. of plasma by 2 mg. per 100 cc. per hour.



BILE PIGMENT METABOLISM IN CHRONIC HEMOLYTIC ANEMIA

FIG. 5. Bile pigment metabolism in chronic hemolytic anemia. With the bone marrow working at full capacity and the anemia stabilized, the amount of hemoglobin destroyed each day should equal the amount produced, and that amount should be constant. The diagram represents the situation in a 70 kg. adult whose marrow is working at the limit of its erythropoietic capacity. Approximately 1,500 mg. of bilirubin are elaborated each day. A failure of correspondence between that figure and the amount of urobilinogen excreted is due to loss of urobilinogen during the process of recirculation or perhaps by excessive dehydrogenation in the colon.

those from other patients with hereditary spherocytosis whom we have examined and with the published results of other investigators.[17,28,29] The daily excretion may range from 400 to 1,400 mg. The amount usually found is about 700 to 900 mg. per day. The reason for the discrepancy between hemoglobin destruction and urobilinogen excretion has not been definitely established but there are several possible explanations. (1) The porphyrin from the degraded hemoglobin molecules may not be converted quantitatively to bilirubin. (2) The bilirubin may not be represented quantitatively as urobilinogen in the feces. In support of the first possibility Grinstein and his associates[8] working with isotope-labelled hemoglobin have found isotopic protoporphyrin in the feces derived, evidently, from this hemoglobin, suggesting that there is at least one alternative pathway, however small, of hemoglobin degradation. There may be others. In support of the second possibility it has been determined that there often exists a wide discrepancy between the anticipated excretion of urobilinogen and the amount actually recovered.[12] It has long been known that a large proportion of the urobilinogen is absorbed from the bowel and some is reexcreted by the liver.[30] During the recirculation some pigment is lost.[12] Constipated subjects excrete much less urobilinogen in their feces than those whose bowels move freely.[23] This has been interpreted to mean that stasis of

feces in the large bowel allows urobilinogen to be absorbed and that much of the recirculated pigment is destroyed. The amount excreted in the urine is quite small. (Figs. 3, 5.)

Other ways may exist whereby the quantitative relationship between bilirubin and urobilinogen excretion is disturbed. There is, for example, a possibility that within the colon itself some of the pigment may be degraded beyond the point where it can be recognized by the reactions for urobilinogen and urobilin. Watson[20] suggests that such pigments as copronigrin and mesobilifuscin may represent bilirubin that has undergone excessive dehydrogenation.

The discrepancy between the rate of hemolysis and the excretion of urobilinogen has been stressed in order to emphasize the significance of the fecal urobilinogen. It is fairly certain that a sustained increase of fecal urobilinogen is diagnostic of hemolytic disease but the quantity of urobilinogen recovered from the stool is not necessarily an accurate index of hemolysis.

The "Cyclical" Excretion of Urobilinogen. The cause of an apparently cyclical pattern of urobilinogen excretion in the patient with nonspherocytic anemia is unknown. (Figs. 2, 3.) It is believed not to be related to a variation in hemolytic activity. The patient's hematocrit remained at a constant level, and although the reticulocyte count varied from 6 to 14 per cent the variations were unrelated to the changes in urobilinogen excretion. It is conceivable that intermittent inhibition of hepatic excretory activity might cause a swing in the rate of urobilinogen excretion in the feces. If this were the mechanism it would be possible also to explain the increased excretion of urinary urobilinogen. If, however, the variations of fecal urobilinogen were due to retention of bilirubin one would expect the plasma bilirubin to rise when excretion slowed. But in this patient the bilirubin remained constant. A constant level of plasma bilirubin existing in the face of variable hepatic excretory activity could only be possible if there were alternative pathways of hemoglobin degradation which varied in activity reciprocally with that of the liver.

The "cyclic" phenomenon was not due to retention of feces. The size of the stools bore no consistent relation to the amount of urobilinogen excreted each day.

The absorption of urobilinogen from the intestinal tract can make a great difference in the total amount that is excreted. It has been estimated that 30 to 70 per cent of the bile pigment passing down the intestine is absorbed from the colon as urobilinogen.[12] This pigment is carried by the plasma to the liver where it is removed and presumably re-excreted. The small amount of urobilinogen that appears in the urine is cleared from the plasma by the kidney during this re-circulation. (Fig. 5.) The urine is not the only channel whereby urobilinogen may be lost from the pathway of fecal excretion. A portion of the total urobilinogen that varies from 0 to 80 per cent cannot be accounted for by analysis of the feces.[12] The amount lost is greater in constipated subjects,[23] suggesting that reabsorption may play some part in the discrepancy between the anticipated urobilinogen and the amount actually recovered. The cyclical variation of urobilinogen in our patient may have been related to reabsorption of the pigment, the periods of low excretion corresponding, perhaps, with periods of intense reabsorptive activity of the colon. The increased amount appearing in the urine at such times (Fig. 3) might then be related to an increased concentration of the pigment in the plasma.

The "cyclical" excretion of urobilinogen—if, indeed, these variations represent a cycle—is a phenomenon that requires further investigation.

SUMMARY AND CONCLUSIONS

1. Hemolytic disease exists when the average survival time of the red cells in the circulation is less than the normal (approximately 120 days). The bone marrow responds to the demands of abnormal hemolysis by producing greater quantities of hemoglobin. The shorter the life span of the red cell, the greater is the requirement for new red cells to maintain in the blood a mass of hemoglobin of normal size. Hemolytic anemia results when the average life span of the red cell becomes so short that the marrow, working at the limit of its erythropoietic capacity, is unable to provide an adequate number of replacements.

2. It is demonstrated that in chronic hemolytic disease, as exemplified by hereditary spherocytosis and hereditary non-spherocytic hemolytic anemia, anemia develops when the average survival of red cells is less than fifteen to twenty days. This implies a production of hemoglobin of approximately 0.6 gm. per kg. of body weight per day. Normal production is estimated to be about 0.09 gm. per kg. per day. The well nourished, hyperplastic bone marrow of these slightly anemic but otherwise healthy men was capable of producing about seven times the

normal output of hemoglobin. It is suggested that the output in sickle cell anemia is slightly less, and it is considerably less in pernicious anemia and Mediterranian anemia.

3. The output of bile pigment is geared to the amount of hemoglobin produced. A normal man producing (and destroying) 6.25 gm. of hemoglobin each day should produce 220 mg. of bilirubin. In chronic hemolytic disease with the bone marrow working at the limit of its erythropoietic capacity to produce 45 gm. per day of hemoglobin, the production of bilirubin should be 1,500 mg. Usually much less than this amount of urobilinogen can be recovered from the feces. This provides additional evidence that bile pigment is not excreted quantitatively as urobilinogen.

4. The results of an extended series of determinations of the fecal urobilinogen in one patient indicate that there may be cyclic variations in urobilinogen excretion.

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Prolonged Treatment of Pernicious Anemia with Vitamin B_{12}*

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Since the isolation of vitamin B_{12} in 1948[1,2] many clinical studies have demonstrated the effectiveness of this substance in overcoming the manifestations of pernicious anemia.[3–7] There is much evidence to suggest that vitamin B_{12} or a closely related compound is the anti-pernicious anemia principle.[7–9] Nevertheless, the question has been raised whether vitamin B_{12} alone is adequate therapy for this disease. It has been suggested by some that patients with pernicious anemia may have a deficiency not only of vitamin B_{12} but of certain other nutritional factors.[10–12] The purpose of the present report is to summarize observations on a large group of patients with pernicious anemia who have been treated only with vitamin B_{12} for periods ranging from one to more than three years.

More than 100 patients with pernicious anemia have been treated with vitamin B_{12} at the Johns Hopkins Hospital.† Many of these individuals had previously received injections of liver extract but others were in relapse and had had no prior therapy. The initial hematologic responses of previously untreated patients were similar to those reported by many other investigators[3–6] and will not be described in detail. However, certain aspects of the early effects of therapy seem worthy of comment.

INITIAL RESPONSE TO THERAPY

The parenteral administration of vitamin B_{12} to patients with pernicious anemia in relapse was followed by changes which were in every way similar to those produced by the injection of potent liver extracts.

Blood Changes. The hematologic response of every patient treated was satisfactory. Seven patients were given a single intramuscular injection of vitamin B_{12} and were then observed without further therapy until maximal effect had occurred. The data of this study are summarized in Table 1. A single injection of 12.5 μg. of vitamin B_{12} produced a striking response in some patients but was inadequate to effect a complete hematologic remission in patients in severe relapse. An injection of 100 μg. seemed to produce a maximal effect. When as much as 100 μg. were given as the initial dose, the response did not appear to be enhanced appreciably by further therapy during the recovery

Table 1*

Patient (Age, Race and Sex)	Vitamin B_{12} (μg.)	Hematocrit before Therapy	Reticulocyte Peak		Hematocrit Peak	
			Per cent	Day	Per cent	Day
W. B. (66, C, M)	12.5	19	25	5	37	26
S. T. (71, C, M)	12.5	12	23	6	23	16
H. L. (46, C, F)	25	16	16	5	33	41
W. K. (82, W, M)	25	35	2	4	41	35
C. E. (57, C, M)	25	30	2	6	32	7
F. L. (62, W, F)	25	17	18	5	34	17
B. C. (58, C, F)	100	22	..	..	40	43

* The response of patients with pernicious anemia in relapse to a single intramuscular injection of vitamin B_{12}. No further therapy was given until it was evident that the hematocrit was no longer rising.

† The crystalline vitamin B_{12} used in these studies was generously supplied by Merck and Co., Inc., Rahway, N. J.

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phase. The leukocyte and platelet counts, low prior to therapy, usually returned to normal before the red cell count and hematocrit reached normal values.

General State of Health. Feeling of well-being often improved dramatically within seventy-two hours following the administration of vitamin B_{12}. Fever, when present prior to therapy, subsided within a few days. (Fig. 1.) Most patients noted impressive improvement of strength within the first week. Five patients displayed evidence of serious mental disturbance before treatment, with defective memory, confusion, torpor and psychotic trends. Remarkable improvement of the mental status of these patients occurred within one week and sometimes within forty-eight hours. The blood pressure of hypertensive patients fell to normal levels during the period of relapse but rose again following the administration of vitamin B_{12}.

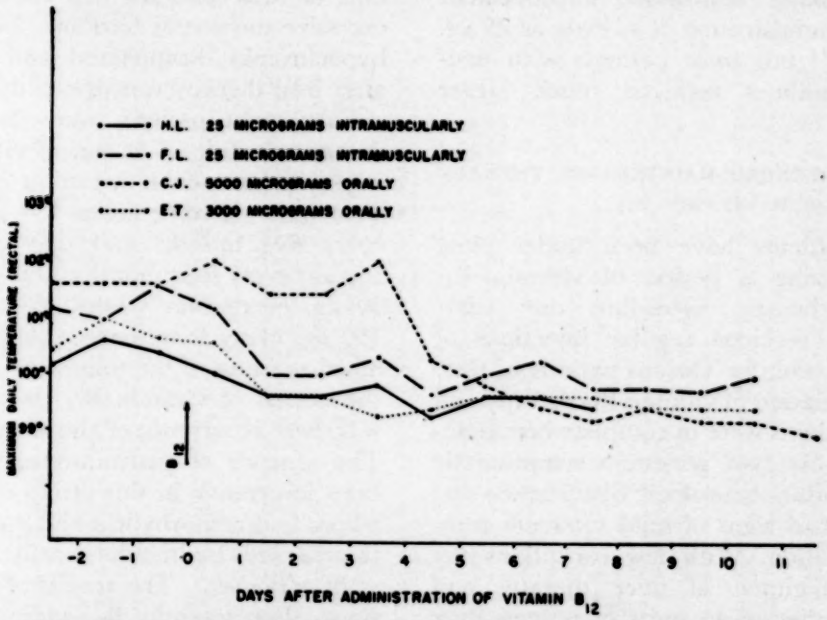

FIG. 1. Effect of the administration of a single dose of vitamin B_{12} on the temperature of patients with pernicious anemia in severe relapse.

Alimentary Symptoms. Soreness of the tongue, when present at the onset of therapy, was noted to disappear within forty-eight hours. Patients who previously had had intermittent soreness of the tongue had no recurrence of this symptom after initiation of vitamin B_{12} treatment. Anorexia, nausea and vomiting were frequently encountered in patients in severe relapse, and subsided within seventy-two hours after vitamin B_{12} was administered. Diarrhea was not a prominent symptom in this group of patients but when present subsided after treatment was begun.

Neurologic Manifestations. Response of neurologic manifestations was quite variable. When symptoms were of short duration and of mild degree, rapid improvement usually occurred. Six patients whose symptoms were limited to paresthesias with only mild sensory impairment were completely relieved of their neurologic abnormalities within two weeks. When neurologic involvement was of longer duration and more severe degree, response to therapy was more erratic. Some patients with severe ataxia associated with loss of peripheral vibration and position sense showed remarkable improvement after small amounts of vitamin B_{12}. On the other hand, some patients showed little or no improvement after very large amounts of the vitamin. A number of patients displayed the manifestations of subacute combined degeneration in the absence of anemia. The normal blood values in these cases sometimes obscured the diagnosis, and in almost every case could be attributed to the injudicious or inadvertent administration of folic acid before the diagnosis of pernicious anemia was established.[13] Of nine such patients studied six showed marked improvement on vitamin B_{12} therapy but all have residual neurologic changes. One patient with rapidly progressing subacute combined degeneration became transiently much worse

during the first week of intensive vitamin B_{12} therapy. This phenomenon has previously been observed at the onset of therapy with refined liver extracts. With the exception of this case, development or progression of neurologic manifestations was not observed during vitamin B_{12} treatment. Striking neurologic improvement followed the administration of as little as 25 μg. of vitamin B_{12}[14] but most patients with neurologic abnormalities received much larger doses.

RESULTS OF PROLONGED MAINTENANCE THERAPY WITH VITAMIN B_{12}

Fifty-four patients have been under close observation during a period of vitamin B_{12} maintenance therapy exceeding one year. Forty-five had received regular injections of refined liver extract for various periods of time prior to the initiation of vitamin B_{12} therapy and all of these patients were in complete hematologic remission. Six had persistent symptomatic evidence of residual neurologic disturbance and several others had signs of mild subacute combined degeneration. With few exceptions the maintenance regimen of liver therapy had been one injection of 45 units of refined liver extract given intramuscularly each six weeks. Liver therapy was discontinued when treatment with vitamin B_{12} was instituted. Nine patients were in relapse when vitamin B_{12} therapy was started and these have had no anti-anemia treatment other than vitamin B_{12}.

Patients in relapse in almost all instances were hospitalized and carefully observed during the period of initial response to vitamin B_{12}. All of the out-patients were followed in the hematology clinic. Physical examination was performed at the time that vitamin B_{12} maintenance therapy was begun and was repeated at intervals of six months or more often when indicated. Patients returned to the clinic regularly for injections of vitamin B_{12}. The blood was examined every three months. An x-ray study of the upper gastrointestinal tract was made each six months in an effort to detect early gastric carcinoma. A number of patients were under treatment for a variety of other unrelated conditions including diabetes, hypertension, cardiac disease, hypothyroidism and prostatic disease. However, no therapy for pernicious anemia was given other than vitamin B_{12}. No folic acid was administered to any patient in this group, nor were other vitamin preparations employed. The patients were encouraged to eat a normal diet and were not instructed to eat liver or any other special foods. Two patients received ferrous sulfate for several months after the initial response to vitamin B_{12} because hypochromia of the red cells was noted. One of these patients was known to have had excessive menstrual bleeding. In both cases the hypochromia disappeared and did not recur after iron therapy was discontinued.

Forty-eight patients were maintained on a dosage schedule of 45 μg. of vitamin B_{12} given in an intramuscular injection every six weeks. Two patients were given 150 μg. by injection every five months and one patient received 150 μg. every four months. One patient received 30 μg. every four weeks and two were given 150 μg. every four weeks. None of the patients noted soreness of the tongue at any time during the period of vitamin B_{12} therapy. In no case was there recurrence of alimentary disturbances. The absence of gastrointestinal symptoms has been impressive in this group of patients, all of whom had achlorhydria and none of whom was treated with hydrochloric acid.

Blood Values. The results of blood examinations after vitamin B_{12} maintenance therapy are summarized in Table II. The duration of therapy was taken as the interval from the beginning of vitamin B_{12} treatment to the time of the blood examination recorded. In no instance has anemia developed. Furthermore, the mean corpuscular volume of the red cells showed no tendency to rise above normal. Careful examination of the stained blood films revealed that moderate anisocytosis was frequently present, although in some cases the smears appeared entirely normal. The presence of occasional macrocytes was often noted but the proportion of macrocytic cells was low. In rare instances a stippled red cell or Howell-Jolly body was seen. The blood of one patient showed a prominent increase in macrocytes with large numbers of Howell-Jolly bodies. The abnormal appearance of this patient's blood has aroused interest since his disease was first treated more than twenty years ago, and these abnormalities have persisted during intensive therapy with various liver extracts as well as with massive doses of vitamin B_{12}.

Neurologic Changes. None of the fifty-four patients maintained exclusively on vitamin B_{12} therapy has shown evidence of progression of subacute combined degeneration. Of the forty-

TABLE II*

Patient	Age, Race and Sex	Duration of Therapy before B_{12} (yr.)	Duration of B_{12} Therapy (mo.)	B_{12} Dosage Schedule (μg.)	Hematocrit at Onset of B_{12} Therapy	Blood Values at End of B_{12} Study Period						
						Hematocrit	RBC	Hemoglobin	MCV	MCH	MCHC	WBC
1. C. M.	(75, W, F)	11	28	45 q. 6 wk.	43.0	44.1	5.4	14.3	82	26	32	5,600
2. J. M.	(80, W, F)	10	32	45 q. 6 wk.	46.2	45.8	5.3	14.0	86	26	31	9,700
3. M. T.	(59, W, F)	1	30	45 q. 6 wk.	40.0	38.3	4.3	11.1	89	26	29	5,200
4. B. S.	(68, C. F)	8	30	45 q. 6 wk.	42.0	45.0	5.7	13.0	79	23	29	6,600
5. A. L.	(60, W, F)	9	30	45 q. 6 wk.	43.3	39.3	4.7	11.7	84	25	30	7,500
6. J. N.	(74, W, M)	9	32	45 q. 6 wk.	44.2	46.2	5.5	15.2	84	29	33	8,500
7. E. S.	(75, W, F)	0.5	31	45 q. 6 wk.	42.5	45.2	5.2	14.4	87	28	32	7,000
8. C. W.	(80, W, M)	0.2	28	45 q. 6 wk.	39.4	41.1	4.8	13.2	86	28	32	6,200
9. G. S.	(62, W, F)	14	31	45 q. 6 wk.	42.4	41.4	5.0	13.5	83	27	33	6,200
10. J. L.	(62, W, M)	12	29	45 q. 6 wk.	43.4	44.8	5.1	14.2	88	28	32	5,900
11. M. S.	(64, W, F)	4	29	45 q. 6 wk.	43.5	47.0	5.6	15.0	84	27	32	7,700
12. G. R.	(64, W, M)	21	30	45 q. 6 wk.	43.0	47.8	5.0	15.2	96	30	32	7,600
13. E. H.	(69, W, M)	22	31	45 q. 6 wk.	40.5	39.3	3.9	12.4	102	32	31	6,400
14. J. D.	(71, W, M)	7	22	45 q. 6 wk.	41.1	42.0	4.8	14.0	89	29	33	6,300
15. A. R.	(77, C, F)	0.3	33	45 q. 6 wk.	37.0	37.4	4.4	11.7	85	27	31	4,200
16. S. S.	(77, W, F)	8	34	45 q. 6 wk.	40.0	42.4	5.0	13.6	85	27	32	6,900
17. A. K.	(61, W, F)	10	30	45 q. 6 wk.	46.0	41.1	4.7	12.9	87	27	31	5,200
18. C. L.	(55, W. F)	10	37	45 q. 6 wk.	50.0	48.6	5.9	15.0	84	26	31	7,100
19. F. M.	(50, C, F)	0.5	40	45 q. 6 wk.	39.0	39.6	4.6	11.5	86	25	29	3,500
20. B. D.	(60, C, F)	4	30	45 q. 6 wk.	41.4	41.0	4.6	12.0	89	26	29	8,800
21. A. P.	(51, W, M)	6	30	45 q. 6 wk.	43.2	47.5	5.5	15.3	86	28	32	5,200
22. N. S.	(38, C, F)	3	29	45 q. 6 wk.	46.2	44.2	5.1	13.9	87	27	31	.....
23. M. D.	(60, W. F)	3	30	45 q. 6 wk.	44.0	43.8	5.3	12.5	83	24	29	8,000
24. T. S.	(47, W, F)	8	30	45 q. 6 wk.	41.0	41.9	5.1	13.0	82	25	31	6,600
25. I. F.	(52, W, F)	2	30	45 q. 6 wk.	42.2	42.0	5.2	13.8	81	27	33	6,000
26. V. F.	(45, C, F)	2	30	45 q. 6 wk.	41.8	40.3	4.6	12.1	88	26	30	10,200
27. A. D.	(67, W, F)	10	21	45 q. 6 wk.	46.0	48.0	...	....	...	..	..	.....
28. D. B.	(54, C, F)	2	31	45 q. 6 wk.	42.4	47.5	...	....	...	..	..	.....
29. B. B.	(78, C, F)	7	33	45 q. 6 wk.	43.0	43.0	5.1	14.0	84	27	33	5,000
30. B. A.	(58, C, F)	12	30	45 q. 6 wk.	39.2	39.9	4.6	12.2	87	27	31	4,500
31. S. B.	(73, C, F)	11	36	45 q. 6 wk.	37.0	40.7	4.7	13.9	87	30	34	5,800
32. R. F.	(48, W, F)	2	26	45 q. 6 wk.	44.0	44.8	5.2	14.5	86	28	32	8,100
33. E. S.	(71, W, M)	1.5	33	45 q. 6 wk.	43.0	43.0	4.9	12.3	88	25	29	5,000
34. A. M.	(53, W, F)	2	32	45 q. 6 wk.	43.8	45.0	5.3	14.7	85	28	33	5,100
35. F. W.	(53, W, F)	9	30	45 q. 6 wk.	42.0	45.0	5.3	14.8	85	28	33	9,600
36. S. Z.	(77, W, M)	1	29	45 q. 6 wk.	46.2	41.0	4.5	13.4	91	30	33	.....
37. J. S.	(69, W, M)	17	30	45 q. 6 wk.	50.6	50.3	5.9	15.5	85	26	31	.....
38. J. G.	(75, C, M)	20	28	45 q. 6 wk.	46.5	38.0	4.2	11.7	91	28	31	5,800
39. W. T.	(53, C, M)	17	30	45 q. 6 wk.	48.0	46.8	4.7	14.7	100	31	31	5,000
40. L. L.	(52, W, F)	18	20	45 q. 6 wk.	41.2	41.0	...	....	...	..	..	.....
41. W. Y.	(62, C, M)	17	14	45 q. 6 wk.	56.0	56.9	...	....	...	..	..	.....
42. E. S.	(38, W, F)	9	20	30 q. 4 wk.	39.9	37.3	4.2	11.9	89	28	32	4,300
43. L. E.	(77, W, F)	13	30	150 q. 5 mo.	39.1	38.0	4.5	12.5	84	28	33	5,400
44. V. M.	(66, W, M)	3	11	150 q. 5 mo.	43.8	46.8	5.0	15.0	94	30	32	6,300
45. N. W.	(72, W, M)	7	28	150 q. 4 mo.	44.0	44.0	5.3	14.5	83	27	33	5,300
46. J. M.	(31, C, F)	..	27 (24) †	45 q. 6 wk.	27.0	40.0	...	....	...	..	..	.....
47. C. B.	(71, W, F)	..	26 (12)	45 q. 6 wk.	22.2	41.0	4.9	13.5	85	28	33	.....
48. J. B.	(48, W, M)	..	25 (24)	45 q. 6 wk.	33.3	47.0	5.0	14.4	94	29	31	9,800
49. W. B.	(66, C, M)	..	28 (28)	45 q. 6 wk.	18.8	46.0	...	....	...	..	..	.....
50. L. H.	(67, C, M)	..	18 (15)	45 q. 6 wk.	15.1	52.5	6.0	16.5	88	28	31	7,300
51. S. T.	(71, C, M)	..	33 (33)	45 q. 6 wk.	12.9	49.0	5.9	15.5	83	26	32	5,300
52. F. T.	(61, C, F)	..	16 (16)	45 q. 6 wk.	16.0	41.8	5.0	12.4	84	25	30	4,200
53. M. L.	(63, W, F)	..	25 (24)	150 q. 4 wk.	20.7	41.4	4.9	12.7	84	26	31	8,900
54. F. W.	(60, W, F)	..	30 (30)	150 q. 4 wk.	43.0	44.0	...	....	...	..	..	4,900

* The results of blood examinations after vitamin B_{12} maintenance therapy are recorded. Patients 1 to 45 had previously been treated with injections of liver extract. Patients 46 to 54 received only vitamin B_{12}. During the initial period of therapy of the latter group larger amounts of B_{12} were usually given than were subsequently used for maintenance. The duration of the maintenance therapy schedule is indicated by the figure in parentheses. †

MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.

five patients who had previously been treated with parenteral liver extract only one has shown neurologic improvement. This patient had improved gradually during the period of therapy with liver, and the manifestations of subacute combined degeneration subsided entirely after treatment with vitamin B_{12} for two and a half years. Other patients whose neurologic symptoms had remained static during prolonged liver therapy showed no improvement when treated with vitamin B_{12}. Of the nine patients who received no therapy other than vitamin B_{12} three had residual neurologic changes after the initial period of therapy. Each of these patients continued to improve over a period of many months.

*Prothrombin Times.** Prolongation of the prothrombin time, not responding to vitamin B_{12}, has been reported to occur in patients with pernicious anemia.[12] Slight prolongation of the prothrombin time was noted in several patients in the present series during hematologic relapse. Following therapy with vitamin B_{12} the prothrombin time returned to normal. The longest prothrombin time encountered prior to therapy was 35 seconds (30 per cent of normal) in a patient with severe anemia. This patient's prothrombin time was normal after twenty-nine months of vitamin B_{12} maintenance therapy. Prothrombin times were determined on the blood of forty-two patients at the conclusion of the study period of maintenance therapy. In every case the prothrombin time was unequivocally normal.

Untoward Reactions to Vitamin B_{12}. Of the fifty-four patients observed during vitamin B_{12} maintenance therapy forty-five had previously been treated with injections of liver extracts. Fifteen of these patients had had one or more significant reactions such as urticaria, fever, substernal oppression, abdominal pain, flushing, hypotension and dermatitis following the injection of liver. A few patients had had many untoward reactions and attempts had been made in several cases to desensitize patients to liver. Liver therapy had been discontinued in one case because of a serious reaction. None of these patients has displayed an untoward reaction to the injection of vitamin B_{12}. Single amounts of crystalline vitamin B_{12} as large as 1,000 μg. have often been given intramuscularly, subcutaneously and intravenously, and in no instance has an undesirable effect been observed.

* Prothrombin times were determined in duplicate by a one-stage method employing rabbit brain thromboplastin which had been carefully standardized so that deviations from the normal were readily detected.

TREATMENT OF PERNICIOUS ANEMIA WITH ORALLY ADMINISTERED VITAMIN B_{12}

Eleven patients with pernicious anemia in relapse were treated by administration of a single oral dose of vitamin B_{12}. Eight of these patients were hospitalized and maintained on a diet markedly deficient in vitamin B_{12} during the period of study. The remaining patients were observed in the out-patient department and were permitted to eat a normal diet. Vitamin B_{12} was administered at least several hours after a meal, and no food was permitted for one hour thereafter. The crystalline vitamin B_{12} was dissolved in a few milliliters of water. The dose ranged from 1,000 to 10,000 μg. In every case there was a striking hematologic response. The reticulocytes reached a maximum concentration on the fourth or fifth day, followed by a rapid rise of hematocrit values. When a single amount of 3,000 μg. or more was given, there was sometimes complete restoration of normal blood values without further therapy. (Table III.) The initial response to these large amounts of vitamin B_{12} was indistinguishable from that previously described after parenteral administration of much smaller doses. There

TABLE III*

Patient (Age, Race and Sex)	Vitamin B_{12} (μg.)	Hematocrit before Therapy	Reticulocyte Peak		Hematocrit Peak	
			Per cent	Day	Per cent	Day
F. T. (61, C, F)	1,000	18.0	21.0	5	28.0	12
L. H. (69, C, M)	2,000	15.1	24.0	5	31.0	19
E. T. (41, W, F)	3,000	14.7	27.6	5	32.0	11
H. J. (65, C, M)	3,000	32.5	5.0	5	39.1	11
A. J. (46, W, F)	3,000	32.0	19.0	4	41.0	28
F. G. (58, W, M)	3,000	17.3	29.2	5	34.3	19
H. W. (68, W, M)	4,000	34.4	7.3	5	51.0	21
B. C. (58, C, F)	4,000	16.9	17.4	5	32.0	19
L. H. (78, W, F)	4,800	21.8	16.0	4	37.8	26
C. J. (63, C, F)	5,000	15.3	40.1	5	36.8	18
P. K. (67, W, M)	10,000	35.8	6.1	5	41.5	10

* The response of patients with pernicious anemia in relapse to the oral administration of a single dose of vitamin B_{12}. No other therapy was given until the hematocrit value remained constant or was decreasing.

was prompt and striking improvement of the general condition, subsidence of fever, rapid disappearance of alimentary symptoms and progressive improvement of neurologic manifestations.

A group of patients with pernicious anemia is being maintained on oral vitamin B_{12} therapy. One patient (C. J., Table III) has received only orally administered vitamin B_{12} for twenty months. After the initial dose of 5,000 μg. this patient was given 1,000 μg. of B_{12} by mouth every four weeks. On this regimen hematocrit values fell to subnormal levels within four months. When the dose was increased to 2,000 μg. every four weeks, the blood returned to normal and has been maintained at normal values. The patient remains in complete clinical remission. One other patient (A. J., Table III) has received no treatment other than orally administered vitamin B_{12}. She has been maintained on 1,000 μg. every four weeks for fourteen months and is in complete clinical and hematologic remission. Fifteen additional patients are receiving 1,000 μg. of vitamin B_{12} orally each week, but the duration of the observation period is too short to determine whether this therapy will be adequate. Prothrombin times were determined on seven patients who have received no therapy other than orally administered vitamin B_{12}. In every case the prothrombin time was normal.

INTENSIVE VITAMIN B_{12} THERAPY OF SUBACUTE COMBINED DEGENERATION

A group of patients with troublesome manifestations of subacute combined degeneration was treated with large amounts of parenterally administered vitamin B_{12}. Three patients had previously been maintained on parenteral liver extract therapy for twenty-two, seventeen and ten years. The neurologic abnormalities in these patients had shown no change for many years prior to the initiation of vitamin B_{12} therapy. Parenteral injection of 150 to 1,000 μg. of vitamin B_{12} at intervals of one to four weeks over a period of five to seven months had no effect on the neurologic manifestations in these cases.

Six patients seen in initial relapse had moderately severe neurologic manifestations. These patients received 150 μg. of vitamin B_{12} by injection at intervals of two to four weeks for eight to twenty-nine months. Five of these patients showed considerable neurologic improvement but the neurologic status of one patient was virtually unaltered. The neurologic response in these patients seemed to be no more rapid and no more complete than that which occurred in other patients receiving only 45 μg. of vitamin B_{12} at six-week intervals.

VITAMIN B_{12} FOR CONDITIONS OTHER THAN PERNICIOUS ANEMIA

Vitamin B_{12} deficiency has rarely been recognized at the Johns Hopkins Hospital in conditions other than pernicious anemia. In one small group of patients megaloblastic anemia has developed following total gastrectomy; observations on these cases are to be reported.[15] No instance of nutritional vitamin B_{12} deficiency has been encountered. Numerous cases of sprue have been studied and megaloblastic anemia has been present in most of these. However, folic acid deficiency has seemed to be the cause of the anemia in these cases. Only one case of sprue has been encountered in which the development of subacute combined degeneration provided clear evidence of vitamin B_{12} deficiency. Other megaloblastic anemias, occurring in pregnancy, in infancy and as a result of nutritional inadequacies, have likewise appeared to be attributable to folic acid deficiency and have responded well to the administration of that substance alone.

Two patients with pernicious anemia-like disease associated with abnormalities of the small intestine have been treated with vitamin B_{12}. Both of these patients had hydrochloric acid in the gastric secretion. In one case extensive resection of the small intestine had preceded the occurrence of anemia and the patient was maintained in complete remission with liver extract and with vitamin B_{12}. The second patient, a fifty-six year old Negro, had megaloblastic anemia associated with moderately severe subacute combined degeneration. X-rays of the small intestine demonstrated multiple diverticula of the jejunum. This patient was initially treated by the oral administration of a single dose of 5,000 μg. of vitamin B_{12}. There was a striking clinical and hematologic response and the blood values returned virtually to normal. The patient was subsequently maintained in remission by the parenteral injection of vitamin B_{12}.

One patient with a bizarre disease is of special interest because his requirement for vitamin B_{12} greatly exceeded the amount re-

quired for maintenance therapy of patients with pernicious anemia. This man had enormous enlargement of the liver and spleen, proved subsequently at autopsy to be attributable to myeloid metaplasia. Free acid was repeatedly demonstrated in the gastric secretion. When untreated, severe macrocytic anemia developed, associated with leukopenia, thrombocytopenia and a megaloblastic marrow pattern indistinguishable from that seen in pernicious anemia. After treatment with liver extract or vitamin B_{12} the anemia entirely subsided, platelets returned to normal levels and the white count rose to about 30,000 per cu. mm. with many immature myeloid cells. At this time smears of aspirated marrow presented a pattern resembling that seen in chronic granulocytic leukemia. This phenomenon was demonstrated on five separate occasions over a period of six years. With the passing years the amount of vitamin B_{12} required to produce this effect progressively increased. Prior to his death from myocardial infarction the patient failed to respond to 45 μg. of vitamin B_{12} per week but anemia was completely controlled by 150 μg. per week. It is conceivable that the high requirement for vitamin B_{12} in this patient was at least in part attributable to increased utilization of the vitamin by the disordered hematopoietic system.

Effectiveness of a Vitamin B_{12} Analog

One patient with pernicious anemia in relapse was treated with a thiocyanate analog of vitamin B_{12}.*,[16,17] This patient was given a single intramuscular injection of 25 μg. of the compound. There was a prompt reticulocyte response and the hematocrit rose from 32 to 43 per cent in twenty-one days without further therapy. The patient was then maintained in complete remission by an injection of 45 μg. of this substance every six weeks for a period of ten months. At that time a supply of the analog was no longer available and the patient has subsequently been treated with vitamin B_{12}.

COMMENT

The parenteral administration of vitamin B_{12} to patients with pernicious anemia in relapse produces a therapeutic response as rapid and complete as that which follows the injection of potent liver extracts. The amount of vitamin B_{12} required to produce this effect is remarkably small. The blood values of some patients in severe relapse return to normal following the injection of as little as 25 μg. of vitamin B_{12} in a single dose. Other patients require a larger amount. However, when as much as 100 μg. are given as the initial dose, the response is usually rapid and complete whether or not additional amounts are given during the recovery period. The administration of a single dose of 25 μg. of vitamin B_{12} to a patient in relapse seems to produce a more rapid and more complete response than has been described during the administration of the same amount at the rate of 1 μg. per day.[18] After vitamin B_{12} has been given, the cerebral dysfunction, alimentary disturbances and state of well-being of patients with pernicious anemia usually show striking improvement before a significant change in the blood has occurred. The response of the neurologic abnormalities is less predictable. Some patients show rapid improvement after small amounts of the vitamin. However, in many patients neurologic improvement is very slow and may continue over a period of many months or occasionally several years. Some patients have permanent neurologic residua. It is difficult, therefore, to determine with certainty the smallest amount of vitamin B_{12} which is optimally effective for treatment of the nervous system disorder. The experience described here suggests that when vitamin B_{12} dosage is sufficient for maximally rapid blood regeneration, neurologic improvement also occurs at a maximum rate. The administration of relatively enormous amounts of vitamin B_{12}, many times the quantity required for optimal hematologic response, did not seem to accelerate neurologic improvement. Other investigators believe that massive dose therapy may be of merit.[19] However, when 1,000 μg. of B_{12} are given intramuscularly in a single injection, virtually 100 per cent of the material appears in the urine within a few hours.[14] It appears unlikely, therefore, that very much of the injected vitamin is utilized. Nevertheless, it is important to remember that when patients with pernicious anemia are inadequately treated the neurologic manifestations may become irreversible. Because of this and because one cannot be sure that small doses of vitamin B_{12} are optimally effective, it is wise to treat patients with neurologic involvement with amounts

* The thiocyanate derivative "MK-50" was supplied by Dr. R. A. Peterman of Merck and Co., Inc., Rahway, N. J.

of the vitamin considerably above that required for satisfactory hematopoietic effect.

At the time that vitamin B_{12} became available a large group of patients with pernicious anemia was under maintenance therapy in the Hematology Clinic of the Johns Hopkins Hospital. Most of these patients were receiving 45 units of refined liver extract by intramuscular injection each six weeks. Many of these patients had been on this standard schedule for years and had received no other therapy. Except for those with irreversible neurologic changes, all of the patients had been maintained in complete hematologic and clinical remission. Since a unit of liver extract was found to correspond in activity to about 1 μg. of vitamin B_{12},[1] it was decided to treat these and other patients with injections of 45 μg. of vitamin B_{12} at six-week intervals. The data which have been provided indicate that this therapy has been effective in maintaining complete remissions in patients treated for as long as forty months. It seems probable that the administration of larger doses at longer intervals may be equally effective, for three patients who received 150 μg. at intervals of four or five months have also remained well. It is noteworthy that two of these patients had previously been satisfactorily maintained on doses of 150 units of liver extract given at the same time intervals, for periods of four and one-half and five years.

A number of investigators have attempted unsuccessfully to treat pernicious anemia with orally administered vitamin B_{12}.[20,21] However, when sufficiently large amounts of the vitamin are given, responses may be obtained which are in every way similar to those produced by parenteral therapy.[14,22] The data presented suggest that about 100 times more B_{12} is required for oral therapy than is required when given parenterally. A study is under way to determine, on a long term basis, adequate dosage schedules for oral maintenance therapy. Until such information has been obtained oral treatment of pernicious anemia with B_{12} should be looked upon as an experimental procedure. The difficulties of establishing that a maintenance schedule is adequate are emphasized by the fact that patients who discontinue therapy altogether may remain in remission for more than two years.[23,24] Most oral preparations commercially available at the present time contain far too little B_{12} to be satisfactory for maintenance treatment of patients with pernicious anemia.

The administration of folic acid to patients with pernicious anemia in relapse is followed by a hematopoietic response. Because of the close interrelationships between vitamin B_{12} and folic acid it appears that an excess of one of these vitamins may decrease the requirement for the other, at least for certain physiologic activities.[25] Some investigators have considered the possibility that folic acid deficiency as well as vitamin B_{12} deficiency may exist in patients with pernicious anemia.[11] The present study provides no support for that view. None of the patients in this series was given folic acid and in no case was there evidence of a need for supplemental supplies of this vitamin. On the other hand, the wilful or inadvertent administration of folic acid to patients with pernicious anemia has sometimes permitted development of severe subacute combined degeneration before adequate treatment with vitamin B_{12} was provided. In a number of instances the folic acid ingested in multivitamin preparations has allowed patients to develop a sometimes confusing picture of subacute combined degeneration in the absence of anemia.[13] Folic acid has no place in the treatment of uncomplicated pernicious anemia. Although it is harmless when administered to patients adequately treated with B_{12},[26] its use is unnecessary in patients receiving a normal diet.

It has been suggested that vitamin B_{12} therapy alone is not adequate for complete maintenance therapy of patients with pernicious anemia. The development of macrocytosis of the red cells has been reported to occur in patients treated only with vitamin B_{12}.[12,27,28] This abnormality was not corrected by the administration of large amounts of vitamin B_{12} or of folic acid and was thought to be related to deficiency of another, as yet unidentified, dietary factor. The diet of at least some of these patients was deficient in beef protein.[28] Owren[12] has reported that patients with pernicious anemia treated with vitamin B_{12} or with refined liver extract have hypoprothrombinemia which can be overcome only by the administration of crude liver extract. The prothrombin deficiency and macrocytosis are thought to be attributable to lack of the same substance.[29] Examination of the blood smears of the patients in the present study showed that the degree of anisocytosis was more marked in most cases than is seen in normal blood. However, macrocytosis was not as pronounced as that described by other

authors.[12,27,28] In only rare instances were the red cells strikingly abnormal and in these Howell-Jolly bodies and stippled cells were present as well as macrocytes. Prothrombin times, determined on the blood of fifty-six patients treated with vitamin B_{12} alone, were normal in every instance. Many of these patients had had no therapy other than vitamin B_{12} or refined liver extract for many years. These data are not in accord with the observations of Owren. Possibly the discrepancy in results is attributable to differences of diet in the two groups of patients.

Present evidence suggests that the only substance in liver extract required for the treatment of pernicious anemia is vitamin B_{12}. There are several reasons why vitamin B_{12} is preferable to refined liver extract for therapy of this disease. It is a pure crystalline substance and is available in much more concentrated form than in liver extract. Therefore, smaller volumes of solution are required and there is considerably less discomfort at the site of injection. Hypersensitivity reactions are not uncommon after the injection of liver extract but do not occur following administration of crystalline B_{12}. Furthermore, the cost of vitamin B_{12} is now much less than that of liver extract.

SUMMARY

A large group of patients with pernicious anemia has been treated with vitamin B_{12} for periods as long as forty months. Parenterally administered B_{12} was as effective as refined liver extract in producing and maintaining clinical and hematologic remission. No evidence was found that patients with uncomplicated pernicious anemia need any therapy other than vitamin B_{12}. Orally administered vitamin B_{12} was effective in the treatment of pernicious anemia provided that the oral dose was about 100 times the amount which was adequate when given parenterally. Until the adequacy of oral dosage schedules has been demonstrated patients with pernicious anemia should receive parenteral therapy. An intramuscular injection of 45 μg. of vitamin B_{12} given every six weeks appeared to be adequate for satisfactory maintenance therapy and protected against hematologic and neurologic relapse. Vitamin B_{12} seems preferable to liver extract in the treatment of pernicious anemia because it causes less discomfort at the site of injection, does not give rise to untoward reactions and is less expensive than liver extract.

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Thrombotic Thrombocytopenic Purpura*

Review of the Literature and Report of Three Cases

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THROMBOTIC thrombocytopenic purpura[13] (thrombocytic acroangiothrombosis,[14] generalized platelet thrombosis,[20] diffuse platelet thrombosis with thrombocytopenia and hemolytic anemia,[16] generalized capillary and arteriolar platelet thrombosis[6]) is a clinical entity concerning which a relatively small but steadily expanding literature exists. Although the basic mechanisms leading to the peculiar lesions and clinical manifestations of this syndrome have yet to be elucidated, the clinical picture is becoming increasingly well defined. Relatively few cases, however, have been recognized antemortem; hence hematologic and metabolic studies are incomplete and rational therapy based on such data is necessarily lacking. It is the purpose of this paper to review the available literature and to add three cases to those already reported. In one of these the diagnosis was correctly made antemortem, various chemical and hematologic studies were carried out and both splenectomy and adrenocorticotropic hormone were employed therapeutically.

REVIEW OF THE LITERATURE

The first description of a case presenting the pathologic and some of the clinical characteristics of this disease was that of Moschcowitz,[1] who in 1925 reported "An Acute Febrile Pleiochromic Anemia with Hyaline Thrombosis of the Terminal Arterioles and Capillaries." His patient was a sixteen year old girl who entered the hospital with an illness of ten days' duration, characterized by weakness of the arms with pain on moving the wrists and elbows, pallor, moderate fever, a café-au-lait tint to the skin and a few scattered petechiae. Liver and spleen were not felt. There was marked anemia and the erythrocytes showed central pallor; moderate leukocytosis was noted. No immature red cells were noted in the peripheral blood and no platelet counts were done. The feces and gastric juice were guaiac-positive. The patient lived one week after admission to the hospital; her course was characterized by fever, the appearance of hemiparesis and a positive Kernig sign, pulmonary edema, coma and death. Autopsy revealed numerous thrombi in the terminal arterioles and capillaries, especially in the heart. These showed varying degrees of organization from the adjacent endothelium associated with a concentric perivascular fibroblastic process. Many lesions were noted also in the kidneys and a few in the liver and spleen. Stains for bacteria, spirochetes and acid-fast organisms were nonproductive Moschcowitz believed that the thrombi were probably composed of agglutinated or rapidly destroyed erythrocytes and he concluded that death was due to "some powerful poison" with "both agglutinative and hemolytic properties."

The condition was not mentioned again in the literature until 1936 when Baehr, Klemperer and Schifrin[2] reported four cases, all in females, with the clinical picture of acute purpura hemorrhagica associated with widespread thrombotic lesions of capillaries and arterioles. Their patients ranged in age from nine and a half to forty-eight years and the duration of the disease from nine days to seven weeks. It was fatal in all cases. Prodromata of listlessness and pallor were noted in two cases; one patient had had mild arthritis for a year before onset and an upper respiratory infection for one week. Another gave a history of transient urticaria one month before onset. Weakness, headache, pallor and purpura marked the sudden onset of the disease. All four patients had fever at some time during their

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course. Clinical icterus was noted in three cases and two patients had café-au-lait or brownish pallor of the skin. The spleen was palpable in one case. The ocular fundi revealed hemorrhages and grayish exudates in all four cases; all four patients had hematuria which was obvious grossly in one. Terminally, evidences of central nervous system dysfunction appeared in the form of irrationality, delirium, stupor, hemiplegia and clonic movements. Splenectomy was performed terminally in one patient but it did not alter the course of the disease noticeably.

Hematologic findings included thrombocytopenia and rapidly progressive anemia responding poorly to transfusions; color index of the red cells was about 1.0. The white blood cells were normal or moderately increased, with a myeloid series shift to the left terminally in three cases. Reticulocytosis and the presence of normoblasts in the peripheral blood were also found.

Pathologically, the bone marrow was normal or slightly hyperplastic; megakaryocytes showed no abnormalities. Splenomegaly was noted at autopsy in one case and in another case there were a few coarse, friable verrucae on the mitral valve which microscopically appeared to be platelet thrombi with some subjacent endothelial and fibroblastic proliferation; no change in the valve substance itself was noted.

The vascular lesions were found in virtually all the organs, the lungs being relatively spared. They were most common in the kidney cortex, adrenal cortex, myocardium and pancreas, and were found in the capillaries and precapillary arterioles. They consisted of granular thrombi with varying degrees of swelling, proliferation and organization by adjacent endothelium. There was no surrounding inflammation and relatively little focal necrosis except in the myocardium. The thrombi were found to stain pink with hematoxylin and eosin: stains for amyloid, iron and hemoglobin were negative. Weigert fibrin stains showed little fibrin in the thrombi. Giemsa stain was used to show the morphology of the agglutinated platelets.

These authors believed that the anemia was due to loss of erythrocytes into the tissues through damaged capillaries and that the destruction of these red cells was responsible for the icterus. They thought that the endothelial changes were probably secondary to the thrombosis and that the various stages of thrombus organization suggested that the lesions occurred in crops. They believed that the supply of platelets was quite adequate in these cases since the megakaryocytes in the marrow appeared normal, and that the number of platelets found in the involved vessels was large enough to have exhausted the supply and thus produced thrombocytopenia. It is of interest that the brother of one of these patients died of purpura hemorrhagica with no vascular lesions; his marrow and spleen were normal.

This report constitutes one of the basic descriptions of the clinical and pathologic features of this disease, and the concepts of the pathogenesis of the thrombocytopenia, in terms of adequate supply from the marrow but excessive "utilization" in the peculiar granular thrombi, contained therein have been widely accepted by later authors.

Friedberg and Gross[3–5,24] described the occurrence of non-bacterial vegetations on the heart valves appearing in a variety of conditions. They reported three cases in which such lesions were associated with an illness characterized by fever and acute thrombocytopenic purpura; case 1 of their report had been previously reported by Baehr, Klemperer and Schifrin,[2] and showed diffuse capillary and arteriolar platelet thrombi at autopsy in addition to non-bacterial vegetations on the mitral valve composed largely of platelets and fibrin. These authors believed that there was some evidence of old, healed rheumatic disease of the valve. The other two patients in their report did not show the characteristic lesions of thrombotic thrombocytopenic purpura at autopsy and probably represented instances of some other entity, possibly disseminated lupus erythematosus.

In 1939 Gitlow and Goldmark[6] reported a case previously described by Baehr et al.[2] and compared it with a case thought to be one of disseminated lupus erythematosus which showed numerous "hyaline masses" and "a suggestion of the so-called wire-loop appearance" in the glomeruli. The small vessels of the other viscera showed no thrombotic lesions but the authors believed that the similarity of the vascular lesions might indicate a relationship between the two conditions. Later authors have not believed that disseminated lupus and thrombotic thrombocytopenic purpura are related, with one exception[27] to be noted later.

Altschule's case,[7] reported in 1942, occurred in a fifty year old woman and was characterized by fever, thrombocytopenic purpura, icterus and a fatal outcome. Marrow examination was

described as normal. The author believed that in view of the differences in the clinical manifestations of the two diseases and in view of the types of vascular lesions found in them, attempts to relate this syndrome to disseminated lupus erythematosus were not valid. He suggested that some damage to the vascular endothelium resulted in platelet deposition; this was the first suggestion that a vascular lesion was primary to the thrombosis in the involved vessels.

The seventh case to appear in the literature was that of Bernheim;[8] this was a thirty-three year old female whose brother had died of periarteritis nodosa. Her illness began shortly after the appearance of dermatitis at the site of application of adhesive tape and ran a fatal course in a little more than two weeks. The bone marrow in this patient was slightly hyperplastic but the differential count was normal. The author pointed out that no endothelial changes were found in vessels which did not contain thrombi and concluded that endothelial swelling and proliferation, when found, were secondary to the presence of the platelet thrombus. It is of interest that postmortem studies on the blood of this patient revealed no agglutinins for platelets of the same blood group. Also of interest was the finding for the first time of numerous platelet thrombi in the cerebral cortex, with associated areas of ischemic necrosis. The author noted, however, that necrosis was not an impressive finding in other organs. She thought this related to incomplete occlusion of involved vessels and to the lack of complete involvement of capillary beds; this idea has been supported by later authors.

The first report of the appearance of the syndrome in a male was that of Trobaugh et al.[10] whose patient was a twenty-four year old man in whom the disease ran its fatal course in fifteen days. From studies with various special staining technics the authors concluded that the thrombi were composed of platelets. They noted also that in involved areas almost all the capillaries showed prominent endothelial cells with vesicular nuclei—even capillaries containing no thrombi. They indicated that such histologic changes might represent the earliest stages of the lesion and that platelet deposition at such sites might follow, as suggested by Altschule.[7] Studies on the bone marrow in this case revealed normal numbers of megakaryocytes, most of which contained dark, pyknotic nuclei. These authors were the first to suggest that the jaundice in these patients might be due to a hemolytic process.

Brown and Norman[11] in 1946 described a young white man who died after a five-year remitting and exacerbating illness characterized by arthritis, fever, abdominal pain, epilepsy, splenomegaly, generalized lymph node enlargement, nephritis with hypertension and terminal azotemia, hyperglobulinemia, one episode of hemorrhagic skin lesions and one episode of mesenteric thrombosis. Early in his course he was not anemic and showed no icterus but the platelet count was 120,000 per cu. mm. During the second year of his illness, during an exacerbation, the hemoglobin was 9.7 gm. per cent but the peripheral blood was reported as otherwise normal. He was found to have mild diabetes at this time. During the final episode there was no anemia and the icteric index was said to be 5; platelet count was 75,000 per cu. mm. Reticulocyte count was 2.8 per cent. He had gross hematuria at this time and was thought to have hemoglobinuria as well.

At autopsy this patient showed widespread purpura. There was terminal acute bacterial endocarditis and some old scarring of the mitral valve. On microscopic examination there were widespread fresh and old "platelet thrombi" in capillaries, arterioles and venules, as well as "vascularized granulation tissue" in the small arteries and veins. The glomeruli showed various degrees of fibrosis. The inflammatory reaction in vessel walls was mild and inconstant. It should be noted that the surgical specimen obtained at the time of his mesenteric thrombosis during his terminal illness showed obliteration of the lumens of many arteries and veins with recanalized granulation tissue and there was mild lymphocytic perivascular infiltration. The central nervous system was not examined at autopsy.

This case presented a clinical course which was at wide variance with that of other reported cases of thrombotic thrombocytopenic purpura. The prolonged course, the prominent joint symptoms, the generalized lymph node enlargement, the absence of clear evidence of hemolytic anemia and the late appearance of thrombocytopenia were atypical features. The lesions in larger vessels, with revascularized thromboses of this type, have not been seen in other cases. The course fits well with that of disseminated lupus erythematosus although the authors specifically state that the lesions did not

resemble those of this disease nor those of periarteritis nodosa. Nevertheless, it would seem possible that this patient suffered from some other vascular disease, perhaps complicated late in its course by thrombotic thrombocytopenic purpura. Possibly some form of diffuse vasculitis existed for several years, on the basis of some type of hypersensitive state, with the lesions of thrombotic thrombocytopenic purpura appearing late as another expression of hypersensitivity. Further, the terminal bacterial endocarditis confuses the clinical and pathologic picture since this disease can produce thrombocytopenic purpura, anemia and embolic lesions which closely resemble so-called "platelet thrombi."[28]

Carter[12] in 1947 added to the literature another case in a male, a sixty-six year old Negro, who died in twelve days of a febrile illness characterized by bizarre neurologic signs of varying intensity in the absence of purpura or clinical icterus. He was, however, anemic with normoblasts in the peripheral blood and had reticulocytosis of 8.5 per cent. Platelets were reduced on smear. The patient showed widespread typical lesions of thrombotic thrombocytopenic purpura at autopsy, including numerous scattered ones in the brain. The marrow revealed abundant megakaryocytes although cytoplasmic details could not be studied due to autolytic changes. Occasional non-thrombosed vessels showed slight endothelial swelling and proliferation. Special staining technics led the author to the conclusion that the thrombi were "probably of platelet origin." Some of the lesions showed marked fibrinoid degeneration of the arteriolar wall, sometimes with actual necrosis and extrusion of the thrombotic material, suggesting that vascular damage is a prominent feature of the syndrome.

Singer, Bornstein and Wile[13] added another case to the literature in 1947 and this patient, an eleven year old white girl, showed, in addition to the classical course and pathologic findings of the disease, two gross subdural hematomas. (Massive intracranial bleeding is uncommon in this condition.) Bone marrow study in this patient revealed increased numbers of megakaryocytes which were normal in appearance. These authors introduced the term "thrombotic thrombocytopenic purpura" and provided an excellent review of the clinical and hematologic features of the disease; they emphasized again that the anemia which characteristically occurs is a hemolytic one.

The three cases of Fitzgerald et al.[14] presented several interesting features: The first, a thirty-four year old man, had severe epigastric pain as a prominent symptom, a relatively common finding. Their second patient, a twenty-seven year old colored woman, had a history of sulfonamide sensitivity manifested by a rash; she also had had recurrent urticaria and an illness diagnosed as acute rheumatic fever. Her terminal illness was ushered in by a bout of urticaria, and on admission she had a macular rash. During her course electrocardiograms showed prolongation of the P-R interval. At autopsy marked thrombotic and purpuric lesions were noted throughout the heart muscle, with rare foci of necrosis. Megakaryocytes were normal or somewhat increased in the bone marrow. The third case was a twenty-four year old white man who had a history of rheumatic fever and had been given a sulfonamide just prior to admission; this was followed by the appearance of a macular rash, epistaxis and red urine. At autopsy diffuse platelet thrombosis was found with necrosis of vessel walls adjacent to some thrombi, with occasional extruded thrombus material. These authors first suggested the name "thrombocytic acroangiothrombosis" for the syndrome.

The case of Engel et al.,[15] a young colored girl with demonstrated hypersensitivity to sulfonamides, ran a course lasting about three months, including a partial remission of about one month's duration, a feature which has not been seen in other cases. Another feature of interest was the prominent cardiac involvement, both clinically and pathologically, the latter including the non-bacterial thrombotic endocarditis described by Friedberg and Gross.[23] In addition to the usual vascular changes of the disease there was some thickening of the basement membranes of the glomeruli. This patient was subjected to splenectomy without alteration of her steadily downhill course. It is to be noted that her general clinical condition had already deteriorated markedly by the time operation was undertaken.

Muirhead et al.[16] presented the case of a fourteen year old white girl who died after a three-week illness characterized by fever, shifting neurologic signs, purpura and hemolytic anemia. Platelet counts on the peripheral blood

of this patient varied widely, the extremes being 87,555 per cu. mm. and 229,600 per cu. mm. Spherocytosis was noted in the peripheral blood in addition to the usual reticulocytosis and normoblastosis. Red blood cell fragility in saline solutions was increased. Splenectomy was performed at a time when the patient was in a terminal state and she died four hours after the operation. Of great interest was the finding in this patient of proliferative glomerulitis, usually not associated with the presence of platelet thrombi in the glomerular capillaries. Endothelial proliferation in the absence of local thrombosis was also found, chiefly in the lungs. The authors believed that some form of antigen-antibody response might explain the pathogenesis of the disease. They believed also that as far as the local lesions were concerned, the evidence for and against primary vascular injury was not conclusive.

The neuropathologic aspects of the condition were reviewed by Adams, Cammermeyer and Fitzgerald,[17] who described in detail the changes in four previously reported cases. Clinically, the chief findings relating to the nervous system in a review of all the cases available in the literature at that time included alteration in the state of consciousness, such as irrationality, confusion, delirium, stupor or coma; also, partial motor paralyses, generalized (but not unilateral or focal) convulsions, agnosia, apraxia and aphasia, nuchal rigidity, flaccid paraplegia, hypoactive or absent deep tendon jerks, hemianesthesia and other disturbances in sensation, with or without motor involvement. The cerebrospinal fluid was described as usually normal.

The chief pathologic findings include the following: endothelial and "probably adventitial" hyperplasia in arterioles, capillaries and venules; platelet thrombi in many of these vessels, especially in cerebral cortex, basal ganglia and brain stem nuclei; foci of nerve cell damage and glial proliferation, as well as scattered petechial hemorrhages. Differential stains led to the conclusion that the thrombi were composed of agglutinated platelets. The authors were unable to decide whether the thrombosis or the endothelial proliferation was the initial change in the involved vessel.

Ehrich and Seifter[19] reported a case in a young colored woman who had been taking an iodine-containing medication. An interesting clinical feature was the appearance of 3 plus glycosuria, correlated at autopsy with extensive necrosis of the islets of Langerhans, in addition to the usual changes of thrombotic thrombocytopenic purpura. The authors believed that this case was due to an antigen-antibody reaction caused by the iodine. While this is a difficult point to prove, it is of interest, as the authors point out, in view of the previously reported association of iodine with periarteritis nodosa[25] and with a serum sickness–like picture.[26]

Green and Rosenthal[20] added two cases to the literature in 1949 and reviewed the case previously reported by Bernheim.[8] Patient 1 of their series had had a smallpox vaccination three weeks prior to admission and rapidly entered into a fulminant picture of thrombotic thrombocytopenic purpura. This patient's marrow showed a normal number of megakaryocytes with normal platelet formation. An interesting feature was the presence of electroencephalographic changes suggesting a neoplasm in the left frontoparietal and temporal regions, associated clinically with aphasia and with weakness and increased reflexes in the right arm; the brain showed marked changes, with capillary thrombi, some gliosis and paucity of cortical cells. No particular concentration of lesions or large gross hemorrhages were noted in the left hemisphere. This patient's cerebrospinal fluid was under normal pressure, was slightly xanthochromic, showed a 4 plus Pandy reaction, protein content of 54 mg. per cent and 2 red blood cells per cu. mm.

Case 3 of this series, a thirty-three year old white woman, showed prominent central nervous system manifestations and also mild abnormalities of the cerebrospinal fluid, with a 1 plus Pandy reaction and a protein content of 61 mg. per cent. This patient also showed mild spherocytosis and some increase in red blood cell fragility in hypotonic saline solution. She was subjected to splenectomy but the course of the disease was unaltered and she died five days after operation. In addition to the usual changes of thrombotic thrombocytopenic purpura, she showed at autopsy foci of "acute pericarditis and myocarditis" as well as kidney changes consisting of occasional obliteration of capsular spaces and fusion of tufts.

Careful studies on the nature of the hemolytic syndrome in this disease were conducted by Singer, Motulsky and Shanberge[21] in a patient in whom the correct diagnosis was made antemortem. This patient was unsuccessfully treated with aureomycin, 2 gm. per day. In addition to

the presence of widespread capillary and arteriolar thrombi at autopsy there were occasional subintimal collections of an amorphous, eosinophilic material, accompanied by endothelial proliferation, with or without a superimposed thrombus.

The anemia in this patient was normocytic; there was reticulocytosis of 10 to 25 per cent throughout the course, and platelets gradually fell from about 33,000 per cu. mm. to almost 0. It is of interest that the patient's red blood cell count could not be raised by transfusion, suggesting that the hemolytic mechanism destroyed the transfused normal cells as well as the patient's own red blood cells. The white blood cell count varied from 5,600 to 12,000, with a shift to the left in the myeloid series; blast cells appeared in the peripheral blood terminally. The proportion of nucleated red blood cells in the peripheral blood also increased during the course of the disease. Spherocytes appeared late and varied in number from day to day.

Red blood cell fragility studies were carried out in a number of media. In hypotonic saline solution there was great day-to-day variation, and on some occasions definite increase in fragility was found; the authors believed that these fluctuations were probably related to the irregular appearance of spherocytes in the peripheral blood. Red blood cell fragility in lysolecithin, usually increased in familial hemolytic jaundice but not in acquired hemolytic anemias, was normal in this patient. Heat (acid) red blood cell fragility, usually increased in paroxysmal nocturnal hemoglobinuria, was normal. Mechanical fragility, which has been reported as increased in various hemolytic anemias, was about twice normal in this case. The Coombs test was negative on three occasions, suggesting that globulin antibodies were not involved in the hemolytic mechanism.

Urinary urobilinogen excretion was never markedly increased, and fecal urobilinogen excretion was 118 mg. per day; it was suggested that the concurrent aureomycin therapy might account for the absence of higher values.

The patient's bone marrow showed erythroid hyperplasia. Fifty-seven per cent of 150 megakaryocytes examined showed platelet production (normal 50 to 86 per cent).

The ultimate pathogenesis of these changes remains thus far unexplained, and the question of whether vessel wall change or thrombosis is primary in the vascular lesion of the disease is also unsettled. Several authors, as noted heretofore, have pointed out endothelial and subendothelial changes in vessels showing no thrombosis in the plane of the particular section. Gore[22] has recently re-emphasized the idea of a primary vascular lesion, subendothelial in location, with secondary accumulation of platelets on a tiny overlying endothelial defect. He added to the literature five cases from the Armed Forces Institute of Pathology; one had complicating sicklemia, one had extensive tuberculosis and one had subacute glomerulonephritis, in addition to the lesions of thrombotic thrombocytopenic purpura. Two cases were said to show no decrease in platelets in the peripheral blood. Gore believed that some sort of hypersensitivity reaction might be involved in the genesis of the disease. Beigelman[27] has concurred in this view, reporting two cases in which, in addition to the lesions of thrombotic thrombocytopenic purpura, features thought to suggest disseminated lupus erythematosus were found. The first case presented periarteriolar concentric fibrosis in the spleen and focal hyaline necroses in the glomerular capillaries; the second case showed more advanced glomerular lesions as well as verrucous endocarditis of the aortic valve. Both patients had had clinical courses which can be considered prolonged and atypical for thrombotic thrombocytopenic purpura alone.

Meacham and his co-workers[29] concluded from studying two cases that vascular damage was the primary lesion in the "thrombosed" vessels and in that sense thought that thrombotic thrombocytopenic purpura was "similar to the so-called collagen diseases." They pointed out the presence of numerous tiny aneurysms in the involved vessels and emphasized the fact that there has been no direct proof that the so-called thromboses are actually composed of platelets. Indeed, they believed, on histologic grounds, that there was strong evidence that "much, if not all, of the material in question represents an intramural degeneration rather than an intraluminal coagulum." In addition one of their cases showed no platelet formation from megakaryocytes in the marrow, suggesting to the authors that the thrombocytopenia in this disease may not be due simply to massive deposition of platelets in small vessels. Case 2 of Meacham's series was of special interest in that the patient had had the disease for at least three years; a two and a half year clinical and hema-

tologic remission followed splenectomy and during her terminal illness adrenocorticotropic hormone seemed to induce an incomplete remission although the dose used was small (20 mg. per day).

CASE REPORTS

CASE I. (L. V., HPPC No. 6647, autopsy No. 12907.) This was the first Johns Hopkins Hospital admission of this fifty-six year old white male fruit inspector who was admitted to the Henry Phipps Psychiatric Clinic on December 9 and died on December 13, 1932. The patient's past general health had been good except for several bouts of renal colic over the twenty years prior to admission. He gave no history of acute rheumatic fever, arthritis, skin lesions or bleeding tendency.

Six weeks prior to admission he cut his hand with a rusty knife. He was treated by his local physician and was given tetanus antitoxin at that time. The cut healed uneventfully. Four weeks before admission he began to complain of recurrent headaches for which he took aspirin. Three weeks before entering the hospital he noted vague abdominal discomfort progressing to severe epigastric pain requiring morphine for relief. There was also eructation, progressive distention, general "nervousness" and profuse sweating. He was noted to cry periodically upon the least provocation. At this time his wife noted yellowish discoloration of his skin. One and a half weeks before entry there was progressive pallor and weakness, with increasing nervousness and melancholy. Five days before admission he had a small epistaxis and a few small hemoptyses. He complained of difficulty with his vision, and numbness and tingling of the left hand and left side of the face; his wife noted transient drawing of his mouth to the left. In the next few days recurrent headaches and progressive loss of interest in his surroundings followed. He frequently mumbled and was difficult to rouse. On the day of admission there was progressive delirium, and he was brought to the hospital.

Physical examination at the time of admission revealed a temperature of 103°F. rectally, pulse 120, respirations 20 and blood pressure 120/70. The patient was a comatose, very pale, well nourished and well developed adult white man who was difficult to rouse and exhibited purposeless movements of the extremities. The tourniquet test was found to be positive when the blood pressure was taken. There were numerous bright red petechiae in the anterior axillary folds and on the upper extremities. He was slightly cyanotic. There were several small, flame-shaped hemorrhages in the ocular fundi, and the media were clouded. The heart was not enlarged, rhythm was regular, sounds were of poor quality and there was a late systolic murmur heard over the base. The lungs were clear but for a few moist rales at the left base. The abdomen was flat, soft and non-tender. The liver edge was palpable one fingerbreadth below the right costal margin; no other abdominal organs or masses were felt. Neurologic examination revealed, in addition to his comatose state, slight left facial weakness shifting to the right later in the day. Both plantar responses were extensor in type but this finding shortly disappeared and the deep tendon reflexes became generally hypoactive. He moved his left arm and leg slightly more than the right.

Laboratory studies revealed the following: Serologic test for syphilis negative; red blood cells 2.69 million per cu. mm.; hemoglobin 6.5 gm. per cent; hematocrit 21.8 per cent; mean corpuscular volume 81 cu. micra; mean corpuscular hemoglobin 24.2 μμg.; mean corpuscular hemoglobin concentration 29.8 per cent; white blood cells 23,300; differential count showed juveniles 4 per cent, polymorphonuclear neutrophils 79 per cent, lymphocytes 15 per cent and monocytes 2 per cent; smear showed marked anisocytosis and poikilocytosis as well as polychromatophilia. There were 4 normoblasts per 100 white blood cells. Urine was clear and amber, with specific gravity 1.020, reaction neutral, 1 plus protein, no reducing substance, foam test for bile negative; sediment of a centrifuged specimen contained 4 to 8 white blood cells and 4 to 8 red blood cells per high power field as well as numerous hyaline and coarsely granular casts. Blood culture was sterile. Serum nonprotein nitrogen was 36 and 45 mg. per cent on two determinations; fasting blood sugar was 120 mg. per cent. Lumbar puncture revealed an opening pressure of 140 mm. cerebrospinal fluid; fluid was crystal clear, Pandy reaction negative, Wassermann test negative, protein 25 mg. per cent, gold curve 0—0. Red blood cell fragility test in hypotonic saline showed beginning hemolysis at 0.50% NaCl, complete in 0.28 per cent NaCl. Van den Bergh reaction (indirect test) 2.0 units; direct test—"direct de-

layed reaction." Weil-Felix reaction was negative as was the stool guaiac test. Clotting time was 7 minutes.

The patient lived only four days after admission, and during this time he ran a fluctuating fever between 99° and 104°F. rectally; there was corresponding tachycardia. He was treated with intravenous infusions of glucose and saline, two transfusions of whole blood and digifoline. He remained comatose, however, with intermittent Cheyne-Stokes respirations. His course was progressively and rapidly downhill and he died quietly on the fourth hospital day. On the day of death (after one transfusion) red blood cells were 2.27; hemoglobin 6.5 gm. per cent; hematocrit 21.7 per cent; red blood cell indices were normal; white blood cells 15,800; differential count showed juveniles 19 per cent, polymorphonuclear neutrophils 60 per cent, eosinophils 1 per cent, basophils 2 per cent, lymphocytes 14 per cent and monocytes 4 per cent. There were 16 normoblasts and 4 myeloblasts per 100 white blood cells; reticulocyte count was 16 per cent; platelets were 70,000 per cu. mm. There was marked anisocytosis and poikilocytosis.

Autopsy four hours after death (Dr. Kindell) revealed the following: The body was that of a well nourished, well developed white man. There was slight scleral icterus as well as a purpuric eruption on the arms and chest. The pericardium contained 200 cc. of orange-colored fluid. The heart weighed 460 gm.; there were numerous punctate hemorrhages scattered through the epicardium. The heart valves were normal. There were numerous punctate hemorrhages in the endocardium. The lungs revealed a patchy consolidation in both lower lobes and a 7 mm. caseous nodule in the right lower lobe. The peribronchial lymph nodes were slightly enlarged and moist. The spleen weighed 120 gm.; the vessels and trabeculae were slightly more prominent than usual. In one portion of the ascending colon were numerous punctate hemorrhages and these were also found in the mucosa of the distal half of the stomach. The pancreas showed scattered, small, old fat necroses. The liver was slightly enlarged (1,820 gm.) and was not grossly abnormal. The adrenals were grossly normal. Each kidney weighed 170 gm. and showed widely scattered punctate hemorrhages over the surface. The kidney capsules stripped easily. There was a 15 mm. hemorrhage under the lining of the pelvis on the right. There were petechial hemorrhages over one vocal cord and in the mucosa of the trachea and larger bronchi. The peritracheal and cervical lymph nodes were moderately enlarged and moist. The bone marrow appeared slightly hyperplastic. Culture of the heart's blood was sterile. The central nervous system was not examined.

Microscopically, there were myriads of small hyaline and granular eosinophilic thrombi in small arterioles, venules and in capillaries in virtually every organ of the body. In some places the vessel wall adjacent to the attachment of the thrombus was defective, occasionally with loss of smooth muscle, but whether this was primary or secondary to thrombosis and organization of the thrombus could not be determined. Some of the thrombi were recovered by a new-formed layer of endothelial cells. Inflammatory reaction in vessel walls and local areas of infarction were inconspicuous, except for a few small patches of fresh necrosis in the myocardium. Lesions were most numerous in the heart, pancreas, stomach, adrenals, kidneys and lymph nodes. In addition the pancreas showed numerous tiny foci of old fat necrosis. The kidneys showed occasional hyalinized glomeruli and arterioles. Patches of necrosis of tubular epithelium were also seen. The spleen showed some increase in periarterial connective tissue but not enough to suggest lupus erythematosus strongly. No "wire loop" lesions were seen in the kidneys. Patchy centrallobular fat accumulations were seen in the liver. Occasional cortical necroses were present in the adrenals.

Comment. It is of interest that this patient had received tetanus antitoxin some two weeks prior to onset of his symptoms. He had a fulminating febrile illness characterized by thrombocytopenic purpura, marked anemia with immature red blood cells in the peripheral blood and icterus, and peculiar central nervous system manifestations. At autopsy there were widespread lesions of thrombotic thrombocytopenic purpura. In addition there were old pancreatic fat necroses which may have been correlated with the severe epigastric pain which was a feature of his illness. A moderate-sized pericardial effusion was also found.

CASE II. (R. R., JHH No. 493875, autopsy No. 21697.) This was the first Medical Service admission of this twenty-one year old white male who was admitted from the Accident Room because of delirium on March 14 and died on March 17, 1949. The patient's paternal grandfather and one sister were said to have had

heart trouble. At the age of seven years he was admitted to the Pediatric Service with acute rheumatic fever with no evidence of cardiac involvement. In 1938 at the age of ten he again had acute rheumatic fever and a year later apical systolic and mid-diastolic murmurs were heard. At the age of fourteen he was again seen and was thought to have acute myocarditis and endocarditis; an aortic diastolic murmur developed over a period of weeks. He had never had signs or symptoms of congestive heart failure.

Two weeks prior to admission he noted the onset of weakness, drowsiness, anorexia and knife-like epigastric pain, with nausea and vomiting. The pain subsided after two days but the nausea and vomiting persisted up to the time of admission. One week before entry severe frontal headache appeared and three days later his family noted increasing pallor and dry cough. For two days before admission he had had alternate chilly and feverish sensations and on the day before admission became delirious.

Physical examination at the time of admission revealed temperature 102.6°F. rectally, pulse 120, respirations 36 (Cheyne-Stokes) and blood pressure 170/70. The patient was a very well developed and well nourished young white man who appeared acutely and desperately ill. He was semi-comatose, thrashing about in bed. The skin had a yellow tint and there were many scattered petechiae and ecchymoses over the body. There were several enlarged lymph nodes in all cervical chains, one in the left axilla, and several shotty nodes in both superficial inguinal chains. Bones and joints were normal. The eyes revealed conjunctival pallor and a patch of old chorioretinitis in the right fundus. Ears, nose and mouth were not remarkable but for pallor of the mucous membranes. The neck was supple. The lungs were clear to percussion and auscultation. The heart was markedly enlarged to the left. The precordium was active and there was an apical systolic thrill. At the apex there was a very loud, harsh systolic murmur as well as a low-pitched, rumbling diastolic murmur; the first sound at the apex was loud and snapping. The second aortic sound was also markedly accentuated and in the aortic area there was a soft, blowing diastolic murmur. No gallop or friction rub was heard. The abdomen was flat and soft, with no palpable organs or masses. Genital and rectal examinations were normal. Neurologic examination revealed no evident motor or sensory loss; all the deep tendon jerks were very active but equal. Plantar responses were flexor bilaterally.

Laboratory studies revealed the following: Serologic test for syphilis negative; Rh positive; red blood cells 2.6 million per cu. mm; hemoglobin 7.5 gm. per cent; hematocrit 23 per cent; icteric index 12; sedimentation rate (Wintrobe) 15 mm./hr. corrected; mean corpuscular volume 88 cu. micra; mean corpuscular hemoglobin 28 $\mu\mu$g.; mean corpuscular hemoglobin concentration 31 per cent; white blood cells 10,800; differential count showed myelocytes 2.5 per cent, juveniles 3 per cent, polymorphonuclear neutrophils 73 per cent; lymphocytes 20 per cent and monocytes 1.5 per cent; there was marked anisocytosis and poikilocytosis, and platelets were reduced on smear. Stool was guaiac-positive. Urine was slightly cloudy and smoky in appearance; specific gravity was 1.008; pH 6; there was no reducing substance present; there was 2 plus protein. Centrifuged sediment contained 5 to 6 white blood cells, about 60 red blood cells and occasional granular casts per high power field. Nasopharyngeal and throat cultures grew normal flora; a petechial lesion was aspirated and cultured, with no growth. Six blood cultures and culture of the sternal marrow were all sterile. Cultures of urine and cerebrospinal fluid were likewise sterile. Blood agglutinin studies for Pasteurella tularensis, Proteus OX2 and Proteus OX19 were all negative. Stool culture grew no pathogens. Blood non-protein nitrogen was 45 mg. per cent, fasting blood sugar 64 mg. per cent and total serum proteins 5.9 gm. per cent, with albumin 4.3 and globulin 1.6 gm. per cent. Total serum bilirubin was 2.5 mg. per cent. Lumbar puncture shortly after admission revealed an initial pressure of 220 mm. CSF; fluid was crystal clear and colorless, and contained 30 white blood cells per cu. mm., mostly mononuclears. Pandy reaction was equivocal, protein content 40 mg. per cent, Wassermann reaction negative and mastic curve 1100000000; sugar and chloride content were normal. Bleeding time on the day of admission was 13 minutes; clotting time (Lee-White) was 8½ minutes.

On admission it was believed that the patient most likely had acute bacterial endocarditis. After the various cultures referred to were obtained, he was placed on penicillin, 2,400,000 units per day intramuscularly, and was given 500 cc. of whole blood. His condition deteriorated rapidly; he continued to have Cheyne-

Stokes respirations and on the first hospital day he had two generalized convulsions. Coma progressively deepened. His temperature remained above 103°F. Repeat studies on the second hospital day revealed blood non-protein nitrogen to be 67 mg. per cent, serum calcium 10.1 mg. and cholesterol 220 mg. per cent. Total serum bilirubin was 4.6 mg. per cent with 1.4 mg. per cent direct reacting. Blood studies at this time revealed a clotting time of 37 minutes (normal up to thirty minutes by this method); prothrombin time was 26 seconds (50 per cent of normal); clot retraction (silicone tubes), none; assay for circulating anticoagulant was negative. Reticulocyte count at this time was 4.4 per cent; red blood cells 1.99 million per cu. mm.; hemoglobin 6.5 gm. per cent; hematocrit 18 per cent; red blood cell indices were normal. White blood cells were 16,600; differential showed myeloblasts 1 per cent, undifferentiated myelocytes 2 per cent, juvenile neutrophils 22 per cent, segmented neutrophils 59 per cent, lymphocytes 10 per cent and monocytes 6 per cent; there was 1 nucleated red blood cell per 100 white blood cells; platelet count was 40,000 per cu. mm; icteric index was 50. Sternal marrow aspiration on this day revealed a cellular marrow; megakaryocytes were numerous but showed no evidence of platelet formation; the proportion of nucleated red blood cells was high but otherwise the differential count was normal. There was marked polychromatophilia, moderate anisocytosis and poikilocytosis. Urine examination on the second hospital day revealed urobilinogen to be present in 1:20 dilution; this was not a fresh urine specimen. Foam test for bile was negative. Electrocardiogram on this day showed sinus rhythm with a rate of 111 per minute; P waves and P-R interval were normal. There was a small Q_3 and the S-T segment in the third limb lead was slightly elevated. T_1 was low, and T_2, T_3 and T_{V5} were inverted. The record was thought to suggest posterolateral myocardial infarction.

Despite vigorous hydration and large doses of penicillin, the patient continued to deteriorate and late on the second hospital day aureomycin (90 mg. intramuscularly every six hours) was added to the regimen, without notable effect on his course. On the third hospital day it was noted that he did not move his right leg at all although the reflexes were described as normal. Respirations had improved somewhat and it was thought that his jaundice was less marked; serum bilirubin was found to be less than 0.8 mg. per cent. Urine urobilinogen was positive 1:10. Transient right-sided twitching of the facial muscles was noted. Repeat lumbar puncture showed the cerebrospinal fluid to be under an opening pressure of 200 mm.; it was pink and opalescent, and contained 2,090 red blood cells per cu. mm. and 2 mononuclear cells per cu. mm. Protein content was 57 mg. per cent.

On the fourth hospital day he showed deepening cyanosis. Non-protein nitrogen had risen to 115 mg. per cent, serum bilirubin was 2.2 mg. per cent, with 0.9 mg. per cent direct-reacting. Total serum proteins were 5.8 gm. per cent, with albumin 4.1 and globulin 1.7 gm. per cent. General downhill course continued with persistent fever and purpura and late on the fourth day he died suddenly, not having regained consciousness since admission to the hospital.

At autopsy (Dr. Robert O'Donnell) there were numerous small petechiae scattered over the body. The pericardial sac contained 150 cc. of a clear yellow fluid. The left pleural cavity contained 500 cc. of bloody fluid. The heart weighed 700 gm. There were numerous small subepicardial and subendocardial hemorrhages. There was marked mitral stenosis and the mitral valve showed in addition extensive, fresh, granular verrucae. The aortic cusps were thickened and adherent; the edges were rolled and smooth and there was a small hyaline vegetation on the posterior cusp. The myocardium was mottled and posteriorly, near the apex, was an area that appeared recently infarcted. The lungs were somewhat heavy and the right middle lobe appeared red and slightly shrunken. The liver weighed 3,300 gm. and showed numerous gray-brown splotches on the surface and on section. The spleen weighed 375 gm. and showed numerous relatively fresh infarcts averaging 4 mm. in diameter. The stomach, duodenum, intestines and pancreas were normal. The kidneys weighed 325 gm. each and were dark red, with "flea-bitten" surfaces. There were occasional tiny subcapsular infarcts on the cut surfaces. The capsules stripped easily. The brain showed softening over the left frontoparietal and right occipital regions. There were a few submucous hemorrhages in the larynx. The eyes showed papilledema. Heart's blood was sterile. The pericardial fluid showed no organisms on smear but on culture grew out an unidentified gram-negative rod, thought to be a contaminant. Smears of the valvular vegetations

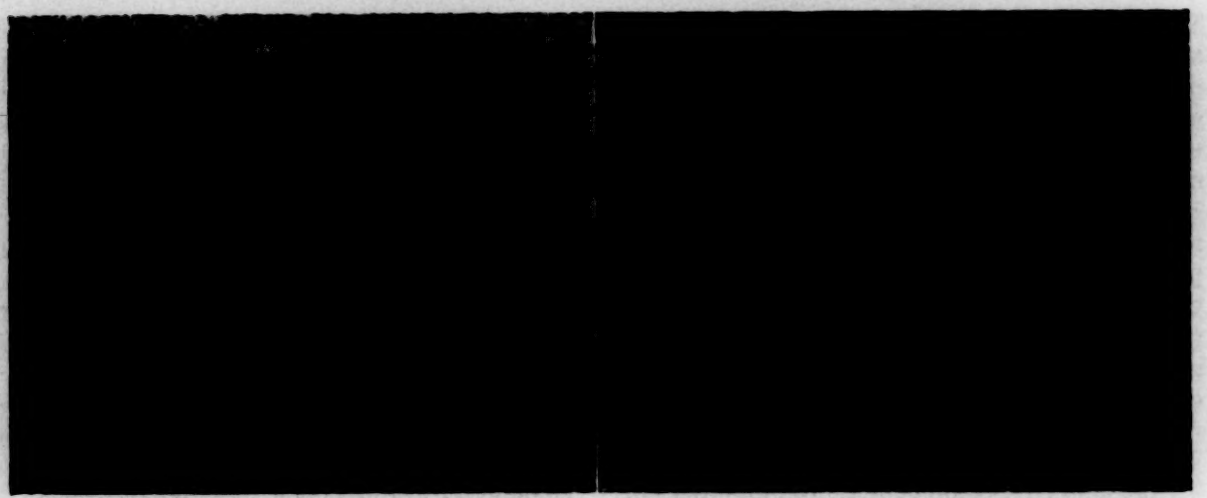

FIG. 1. Case II. "Platelet thrombi" in small renal vessels; × 150.

FIG. 2. Case II. Splenic arterioles showing marked perivascular fibrosis; × 150.

showed no organisms; cultures grew Escherichia freundii, thought to be a contaminant or post-mortem invader.

Microscopically, throughout the heart, lungs, lymph nodes, pancreas, liver, kidneys, spleen, brain and adrenals there were innumerable eosinophilic, hyaline or granular thrombi in arterioles, capillaries and venules, with little or no alteration of the adjacent vessel walls. (Fig. 1.) In the myocardium were numerous tiny necroses and fresh scars as well as a large recent infarct in the posterior wall of the left ventricle; the mitral valve showed interruption of the endothelial lining by a hyalinized mass of connective tissue which was prolonged in one section to adhere to a mass of hyalinized debris containing a moderate number of leukocytes, mostly polymorphonuclears. Bacterial stains of the myocardium and vegetations showed no organisms. The sections did not show a large occluded coronary artery to account for the posterior myocardial infarction although such an occlusion must have been present; it seems likely that an embolus from the valvular vegetations produced the infarction. The liver showed considerable patchy central lobular necrosis with surrounding fatty and cloudy degeneration. The thrombi in the liver were found chiefly in the septal arteries and capillaries. The spleen showed areas of necrosis and marked periarterial fibrosis indistinguishable from that seen in disseminated lupus erythematosus.[28] (Fig. 2.) Although lesions were seen in the kidneys that suggested so-called "wire loop" lesions, none of these were sufficiently striking to be regarded as definite. There were occasional areas of tubular degeneration in the renal cortices. Careful search of the available sections revealed no other lesions strongly suggestive of disseminated lupus. The pancreas and lymph nodes showed a few small areas of necrosis. Numerous thrombi were seen in the cerebral cortex but there was almost no surrounding necrosis. Sections of spinal cord showed occasional thrombi in small pial vessels.

Comment. This patient had a well documented history of recurrent acute rheumatic fever and rheumatic heart disease. He presented a clinical picture which was thought to represent fulminating bacterial endocarditis but numerous blood cultures were sterile and the valvular vegetations showed no organisms on smear at autopsy. There were widespread lesions of thrombotic thrombocytopenic purpura at autopsy in addition to old rheumatic heart disease and lesions in the spleen which were quite characteristic of disseminated lupus erythematosus. An additional finding of interest was a large myocardial infarction, thought to be due to coronary embolization from the valvular vegetations. The patient presented nothing clinically to suggest that he had disseminated lupus, although thrombocytopenic purpura and hemolytic anemia are occasionally seen in that disease.

CASE III. (F. S., JHH No. 247503, autopsy No. 22905.) This was the third Johns Hopkins Hospital and first Medical Service admission of this forty-two year old white housewife who was admitted on February 23, 1951, through the Accident Room because of numbness of the extremities of one month's duration, and who

died on March 8, 1951. Family history revealed that one sister had died of an unknown type of heart and kidney disease. There were no familial bleeding tendencies. Past history revealed good general health. For many years she had noted evening ankle edema but had never had dyspnea, orthopnea, paroxysmal nocturnal dyspnea or chest pain. She had had two prior admissions to this hospital for spontaneous deliveries; she had mild pre-eclamptic toxemia with both pregnancies. About one month before the present admission she began to note sensations of numbness in the hands, feet and legs, associated with mild general fatigability. Her family began to note subtle changes in her personality which eventually advanced to become full-blown paranoid ideas. In the few days prior to admission she had noticed the spontaneous appearance of small red spots and bruises over her body. She had been taking aromatic spiris of ammonia at home as well as an unknown type of "nerve pill."

Physical examination on admission revealed temperature 100.4°F. rectally, pulse 92, respirations 18 and blood pressure 112/80. The patient was an obese, withdrawn, quiet woman who appeared neither acutely nor chronically ill. There was no cough, dyspnea or cyanosis. There were scattered areas of vitiligo over the trunk, shoulders and in the axillas. There were scattered petechiae and ecchymoses over the arms and a few were also seen on the abdomen. The eyes revealed several superficial hemorrhages and one Roth spot in the left fundus. There was a small crust of blood on the right side of the nasal septum. The gums were spongy, with one small bleeding point. The neck was supple. The lungs were clear to percussion and auscultation. The heart was normal. The abdomen was obese, soft and non-tender. The liver was not palpable; the spleen descended two fingerbreadths below the left costal margin on inspiration and was firm and non-tender. Neurologic examination revealed that she was markedly withdrawn; some of her movements suggested catatonia. She was fairly well oriented; the right knee jerk was slightly more active than the left but other than this there were no lateralizing signs. Plantar responses were flexor bilaterally.

Laboratory studies were as follows: Serologic test for syphilis negative; the red blood cell count, 3.55 million per cu. mm.; hemoglobin 9.7 gm. per cent; hematocrit 30.7 per cent; icteric index 11; sedimentation rate (Wintrobe) 25 mm./hr. corrected; mean corpuscular volume 87 cu. micra; mean corpuscular hemoglobin 27 μμg.; mean corpuscular hemoglobin concentration 32 per cent; white blood cells 3,400; differential count showed blast cells 2 per cent, myelocytes 9 per cent, juveniles 21 per cent, polymorphonuclear neutrophils 33 per cent, eosinophils 3 per cent, basophils 1 per cent, lymphocytes 15 per cent and monocytes 16 per cent. Smear showed marked reduction of platelets, slight polychromatophilia and anisocytosis. There were occasional nucleated red blood cells. Blood non-protein nitrogen was 38 mg. per cent, fasting blood sugar was 104 mg. per cent, total serum bilirubin was 2.5 mg. per cent with 1.0 mg. per cent direct-reacting. Lumbar puncture revealed an opening pressure of 140 mm. of cerebrospinal fluid, with normal dynamics; fluid was clear and colorless, and contained 2 white blood cells per cu. mm.; the protein content was 75 mg. per cent; Wassermann reaction was negative. Three examinations of the peripheral blood for "L.E. cells" were negative. Urine and blood cultures were sterile. Total serum proteins were 7.3 gm. per cent, with albumin 4.2 and globulin 3.1 gm. per cent; cephalin flocculation was 3 plus; thymol turbidity was 6.5 units. Examination of the blood for bromides was negative. Serum amylase was 143 mg. per cent; prothrombin time was 24 seconds (approximately 50 per cent of normal). Platelet count early in her course was 58,000; reticulocyte count was 14.8 per cent. The patient's blood group was OMN Rhl Hr′ negative. Serum showed no Rhl, Hr or auto-antibodies in albumin, serum and saline media at room temperature and at 37 degrees. Cold antibody was present in all three media at 4°C. but was not titered. Red blood cells were negative for adsorbed antibodies with serum, albumin and albumin-serum media at room temperature and at 37°C. Direct Coombs test at room temperature was negative. It was concluded that there was no evidence of iso-or auto-sensitization and no evidence of attached antibody on the red cells.

Sternal marrow aspiration, done on the second hospital day, revealed cellular marrow smears. Numerous megakaryocytes were seen but none were producing platelets. The erythrocytic series was increased, with many polychromatic and basophilic normoblasts. The red blood cells showed some polychromatophilia.

FIG. 3. Case III. Subendocardial petechiae and gross hemorrhages.

Electrocardiograms showed only sinus tachycardia on admission, and except for slight general decrease in amplitude, were unchanged six days later.

The patient did not appear generally seriously ill on admission, except for fluctuations in her mental state. She showed alternately a markedly withdrawn and suspicious attitude and a very pleasant and cooperative one, and in addition she had episodes of excitement and combativeness. On her second night in the hospital there was a transient episode of aphasia associated with numbness of the right arm and leg. Throughout the first week in the hospital increasing amounts of sedation were required because of increasingly psychotic behavior. On the eighth hospital day bright red blood was noted mixed with the patient's stools.

In view of the fact that she had run irregular low grade fever throughout her first week in the hospital, associated with marked central nervous system manifestations, thrombocytopenic purpura and hemolytic anemia, the diagnosis of thrombotic thrombocytopenic purpura was suggested by Dr. C. L. Conley. It was decided to transfuse her rapidly and to undertake splenectomy while her general clinical condition was good. Accordingly, she was given 1,500 cc. of whole bank blood on the seventh and eighth hospital days, and on the eighth day splenectomy was performed under sodium pentothal anesthesia. Just prior to operation the platelet count was 30,000 per cu. mm., hematocrit was 34.5 per cent and reticulocyte count was 9.2 per cent; icteric index was 18. Bleeding at operation was not excessive; the patient received 500 cc. of whole blood during the procedure and 500 cc. immediately afterward. About ten minutes after the end of the operation it was noted that the left pupil was widely dilated although it still reacted to light. Four hours later both pupils were equal and reacted well to light. Seven hours postoperatively the right pupil was widely dilated, the patient was moving the left side less than the right and there was flaccidity of the extremities on the left. Deep tendon reflexes on the left were unobtainable and there was a questionably extensor plantar response on the left. It was thought that vascular lesions had probably developed in the mid-brain.

She received 250 cc. of fresh blood by direct transfusion in the immediate postoperative period. Four hours after splenectomy platelet count was 22,000 per cu. mm. and reticulocytes were 9.4 per cent.

On the day following operation she was awake but somewhat disoriented; her general condition was good. Pupils were equal at this time and reacted normally to light. She was given another 300 cc. direct transfusion. Platelet count was 18,000 per cu. mm. and reticulocytes were 18.4 per cent. High fever had appeared, her temperature at one point reaching 105.2°F.

During the second postoperative day high fever persisted and she was very lethargic and disoriented. Gross bleeding from the gums and bowel had appeared. Her hematocrit had dropped from 33.5 to 26.8 per cent in twenty-four hours, and she was given 1,000 cc. of bank blood. Platelet count on this day was 14,000 per cu. mm., reticulocytes were 20.8 per cent, and there were 32 nucleated red blood cells per 100 white blood cells. White blood cells were 15,700, with 2 per cent differentiated myelocytes, 18 per cent juvenile neutrophils, 58 per cent segmented neutrophils, 12 per cent lymphocytes and 10 per cent monocytes. Bilirubin was 3.5 mg. per cent, with 2.0 mg. per cent direct-reacting.

On the third day after operation, with the patient's general condition deteriorating rapidly, her purpura increasing and with the appearance of generalized convulsions, it was decided to add adrenocorticotropic hormone (ACTH) to the therapeutic program. She was given 40 mg. intravenously in 750 cc. of 5 per cent glucose over an eight-hour period, and in addition was started on 40 mg. intramuscularly every six hours. There was no evident effect on

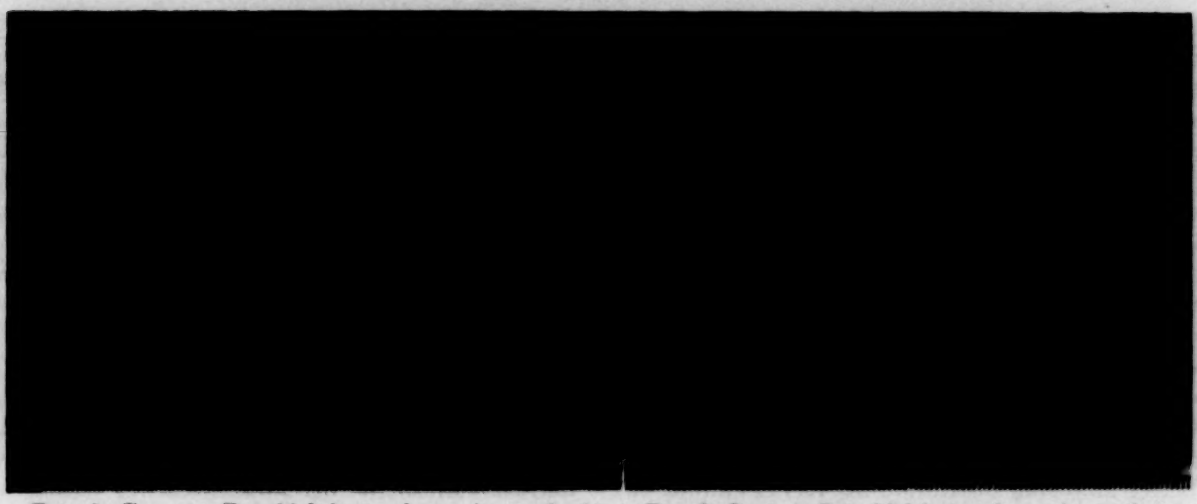

FIG. 4. Case III. Petechial hemorrhages in cerebral cortex and basal ganglia.

FIG. 5. Case III. Petechial hemorrhages in cerebellum and brain stem.

the course of her disease although the peripheral blood eosinophils did not fall appreciably on this schedule. She became comatose, her neck became stiff and she continued to have occasional generalized convulsions. She died quietly on the fourteenth hospital (fifth postoperative) day. Repeat blood studies on the day before death revealed a platelet count of 22,000 per cu. mm., reticulocyte count of 8.2 per cent, white blood cells 10,900 with persistent marked shift to the left in the myeloid series and 58 nucleated red blood cells per 100 white blood cells; there were a few spherocytes present on smear. Red cell fragility studies in hypotonic saline revealed beginning hemolysis in 0.57 per cent saline, well marked hemolysis in 0.48 per cent and complete hemolysis in 0.30 per cent; corresponding control figures were 0.48, 0.45 and 0.30 per cent.

At autopsy ten hours after death (Dr. Terence Cochrane) the body was that of a well nourished and well developed white woman. The skin and mucous membranes were slightly icteric. There were numerous petechiae over the upper extremities. There was a recent splenectomy incision in the left upper quadrant of the abdomen. The abdominal cavity contained 150 cc. of blood; the left pleural cavity contained 200 cc. and the pericardial cavity 150 cc. of slightly icteric fluid. The heart weighed 360 gm.; there were petechiae scattered over the epicardium and the endocardium showed subendothelial hemorrhages in all four chambers. (Fig. 3.) The myocardium was brown and uniform in color. The edges of the mitral valve were slightly thickened and there was a small hyaline vegetation on this valve. The lungs showed mottled dependent congestion and a few petechiae scattered over the cut surfaces. The liver weighed 1,850 gm.; in the porta hepatis was a 2 cm. lymph node with a hemorrhagic center. The stomach, duodenum, small bowel and colon showed many submucous petechiae. The pancreas showed several small areas of fat necrosis in the tail. The adrenals were normal in size and showed several petechiae scattered throughout the cortices. The kidneys were large and showed numerous petechiae throughout the cortex, medulla and calyces. There was a large hemorrhage in the left perirenal fat. There were petechiae also in the mucosa of the bladder and cervix, in the pharyngeal mucosa and in the diaphragm. There was a long clot in the uterine cavity. The bone marrow looked hyperplastic grossly. The brain weighed 1,180 gm. The meninges were delicate and the superficial cerebral vessels were markedly distended. On coronal sections tiny petechiae were found scattered diffusely in the gray matter of the cerebral cortex, basal ganglia, cerebellar cortex and dentate nuclei. There were no hemorrhages over 1 mm. in diameter. (Figs. 4 and 5.)

The spleen (surgical pathology No. 51–919) weighed 410 gm. It was soft and showed prominent malipighian bodies grossly. No discrete lesions were identified.

Microscopically numerous fresh and organizing hyaline and granular eosinophilic thrombi were found in heart, lungs, spleen, kidneys, adrenals, brain, tongue and lymph nodes. There was relatively little alteration in the walls of the involved vessels although occasionally,

underlying a thrombus, local defects in vascular musculature were seen. Except in the myocardium, necrosis was surprisingly slight. The spleen and kidneys showed no lesions suggesting disseminated lupus. Thrombi in the brain were found especially in the cerebral cortex, basal ganglia and cerebellum. Occasional lesions in the skeletal muscle were found.

Comment. This patient's disease was diagnosed antemortem and splenectomy was undertaken while her general clinical condition was still good; there was no evident beneficial effect from this procedure. ACTH therapy was begun at a time when she was moribund and for this reason the absence of change for the better following institution of ACTH cannot be construed as evidence that the drug is of no benefit in this condition. Of considerable interest is the finding of megakaryocytes which showed no platelet production, both in Case II and in this case. This would suggest that decreased supply of platelets from the bone marrow may be a factor in the thrombocytopenia in this disease. Also of interest in this patient was the fact that examinations of the peripheral blood for "L.E. cells" were negative.

REMARKS

Further progress in understanding the pathogenesis of thrombotic thrombocytopenic purpura and in treating it rationally will depend on antemortem recogniton of the disease. It should be suspected when patients present with fever, thrombocytopenic purpura, evidences of hemolytic anemia (icterus, anemia, immature red blood cells in the peripheral blood and increased fecal and urinary urobilinogen excretion), and bizarre and varying central nervous system manifestations. Frequently hepatosplenomegaly will be found. Other features may be abdominal pain, listlessness, a café-au-lait tint to the skin and pallor. The cerebrospinal fluid may be normal or may contain slightly increased amounts of protein as well as red and white blood cells. Individual organ involvement is reflected clinically most clearly in the case of the brain; in one instance[19] glycosuria was associated with extensive lesions in the islets of Langerhans. Cardiac abnormalities may reflect the thrombotic lesions with associated small myocardial necroses or may be due to associated rheumatic heart disease. In one case in this series a large myocardial infarction was evidently associated with coronary embolization from non-bacterial valvular vegetations. Serum proteins have been normal in the cases in which they were determined.

The blood picture is characterized by a normocytic normochromic anemia which tends to progress as the disease advances, and which is only very transiently benefited by transfusions; there are abnormal numbers of reticulocytes and nucleated red blood cells in the peripheral blood. There is thrombocytopenia associated with prolonged bleeding time, poor clot retraction and positive tourniquet test. White blood cells may be normal or increased and the differential may or may not show immature cells of the myeloid series. On occasion spherocytes may be found in the blood and red cell fragility in hypotonic saline may be increased. Sternal marrow may show a normal differential count or there may be erythroid hyperplasia. In most reports normal platelet production from megakaryocytes is noted but in two of the three patients in this series no platelet production was evident and the same was true of one of the cases of Meacham et al.[29]

The disease has been fatal in every recorded case; in one case there was a three-month course with a partial remission of about one month's duration,[15] and in another a three-year course,[29] but the other cases have run steadily downhill courses in from two to nine weeks. The case of Brown and Norman[11] is an exception but this patient presented so many atypical features and such an unusual course that it is difficult to categorize it properly.

The true nature of the disease remains obscure. It has been assumed that the tiny thrombi are composed of blood platelets, and this may be so although it has not been directly demonstrated as yet. Three of the thirty-two patients now in the literature gave a history of acute rheumatic fever or had rheumatic heart disease at autopsy. In addition Beigelman[27] presented two patients who were thought to have histologic lesions of disseminated lupus erythematosus, and one of the cases in the present series had marked periarterial fibrosis in the spleen; there is a history of sulfonamide sensitivity in three of the cases in the literature and two patients had a history of urticaria. One had lesions of subacute glomerulonephritis in addition to those of thrombotic thrombocytopenic purpura. There is some overlap in these associated findings but, in all, eight patients (25 per cent) of the thirty-

two now reported had evidences of conditions thought by some to be diseases of hypersensitivity in addition to thrombotic thrombocytopenic purpura. It may well be that this syndrome is a manifestation of a hypersensitive state and it may be related, in some cases at least, to disseminated lupus. It seems increasingly likely that vascular injury is an important feature of the lesions. It would seem that studies along these lines might be profitable in future cases; perhaps therapeutic trials on ACTH or cortisone might be given earlier in the course of the disease than was possible in our patient or in the patient of Meacham et al. It will be of value to search peripheral blood and bone marrow for "L.E. cells." Further studies on the nature of the hemolytic mechanism are indicated. Evaluation of splenectomy as a therapeutic procedure is as yet premature since only seven have been performed in this syndrome and four of these patients were terminally ill when the operation was done. Splenectomy may have been responsible for the long remission in Meacham's patient.[29]

SUMMARY

1. The literature on thrombotic thrombocytopenic purpura is reviewed and three cases are added; in one of these the patient was unsuccessfully treated with splenectomy and ACTH.

2. Eight of the thirty-two patients now reported have shown, in addition to thrombotic thrombocytopenic purpura, evidence of some condition thought to be due to a hypersensitive state. The possibility that thrombotic thrombocytopenic purpura may be a manifestation of a hypersensitive state or even, in some cases, of disseminated lupus erythematosus, is discussed.

3. The importance of further study of the condition and of increased efforts at antemortem diagnosis is stressed.

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Addendum. Since this paper was submitted for publication, an additional case has been recognized during life and studied by Doctor Thomas Green and his associates. Preparations of this patient's peripheral blood were negative for "L.E. cells."

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Incidence of Leukemia in Survivors of the Atomic Bomb in Hiroshima and Nagasaki, Japan*

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THE concept of irradiation as a leukemogenic agent is not a new one. A considerable amount of experimental work in animals has been done concerning the effects of irradiation and its possible relationship to the development of leukemia. There is general acceptance of the experimental evidence that leukemia may be produced in susceptible species of animals by exposure to roentgen irradiation.[1] Reports suggesting that man may be similarly affected have appeared since 1911 but the evidence has been less convincing.[2] Martland in 1931 reported agreement among authorities that contact with radioactive substances and x-rays produces alterations of which the principal objective symptoms are leukopenia, more rarely a leukemia, and an anemia of the aplastic type.[3] Recently, March has reported that leukemia has occurred as a cause of death more than nine times as frequently in radiologists as in non-radiologic physicians in the United States.[1]

In 1948 the Atomic Bomb Casualty Commission initiated the first survey of the incidence of leukemia in whole human populations exposed to high energy radiation by the explosion of an atomic bomb. The aim of the investigation has been to obtain information concerning all individuals in Hiroshima and Nagasaki having onset of symptoms of leukemia or dying of the disease since the atomic explosion in 1945. It was found that data previous to late 1947 were unreliable and insufficient due to the destruction of records and the general medical conditions prevailing.

The purpose of this report is to present data on the incidence of leukemia and deaths from leukemia in the survivors of the bombing in Hiroshima and Nagasaki during the years 1948, 1949 and 1950 and to compare the incidence and death rate from leukemia in individuals exposed to radiation at various distances from the hypocenter.

CASE MATERIAL

In all cases of leukemia from all sources an attempt has been made to establish the location of the individual at the time of the explosion of the atomic bomb, to determine the presence or absence of symptoms of radiation injury and to confirm the diagnosis of leukemia by objective criteria. The sources of material in Hiroshima were (1) patients referred by physicians to the Commission with the diagnosis of leukemia or suspected leukemia; (2) patients admitted to hospitals of the city with the diagnosis of leukemia or suspected leukemia; and (3) cases discovered by a review of the death certificates on file with the Health Center of the city in which leukemia was listed as a cause of death.

The patients seen at the Commission's laboratory were given complete medical histories, including a history concerning symptoms of radiation injury, complete physical examina-

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tions, peripheral blood studies and, in many cases, bone marrow aspirations or biopsies.

Patients in outside institutions were examined by the Commission's personnel whenever possible. In all cases the hospital records of this group were reviewed and blood smears and bone marrow preparations, when available, were examined.

Cases which were discovered by death certificate were investigated by means of contact with the immediate family, the physician of the deceased and, in patients who had been hospitalized, the review of hospital records, laboratory data and autopsy material. Information obtained from death certificates was personally checked by the Commission.

Data on the cases of leukemia in Nagasaki were largely obtained from investigation of individuals dying from the disease since January, 1948. The initial source of material was death certificates on file in the Nagasaki Health Center. The same procedure of investigation was followed as in Hiroshima.

The Commission investigated a total of ninety cases of leukemia. Insufficient information concerning possible exposure to the atomic bomb, residence in another city and onset of symptoms of leukemia previous to August, 1945, eliminated six of this total number. Table I shows the distribution of these eighty-four cases and the deaths from leukemia in each group.

Table II lists the forty-seven exposed cases of leukemia with the method of diagnostic confirmation available. In only four cases located by means of a death certificate could no objective confirmation of the diagnosis be made. The entire series of cases is available for analysis by age and sex distribution and the type of leukemia. However, only those patients having onset of symptoms or dying of the disease during the years 1948, 1949 and 1950 with residence in the cities of Hiroshima and Nagasaki have been used in the analysis of the incidence of leukemia as it is for this period of three years in these cities in which data are complete.

TABLE I

ACCEPTED CASES OF LEUKEMIA INVESTIGATED BY THE COMMISSION IN THE EXPOSED AND NON-EXPOSED POPULATION AND THE NUMBER OF DEATHS IN THESE GROUPS

	EXPOSED		NON-EXPOSED	
	Cases of Leukemia	Deaths from Leukemia	Cases of Leukemia	Deaths from Leukemia
Hiroshima	31	22	23	19
Nagasaki	16	15	14	14
Total	47	37	37	33

POPULATION DETERMINATIONS

The incidence of leukemia in the exposed population was computed on the basis of the total numbers of that population. The figures for the exposed population presently residing in the cities of Hiroshima and Nagasaki used in the incidence calculations of this report were obtained from the Commission's survivor questionnaire circulated with the Japanese National Census of October 1, 1950. The accuracy, within 10 per cent, of the figures from this source has been confirmed by population estimates obtained from the Public Health and Welfare Section of SCAP, a sample census by the Commission in 1950 and the Commission census file.

Distribution of the exposed population of the two cities by distance from the hypocenter was established by a radiation census in which the location of the individual at the time of the bombing was determined by trained interviewers.

COMPARATIVE INCIDENCE

Table III shows the incidence of leukemia in all of Japan and the United States for 1940. Data for Japan are not available for the war years and the immediate postwar years of 1941 through 1947. No data for the cities of Hiroshima and Nagasaki are available as the records were destroyed in 1945.

It becomes apparent from a comparison of the available statistics in Japan with those of the United States that the reported incidence of leukemia in Japan averages only one-third of that in the United States in terms of per million living population and about one-fourth in terms of per 10,000 total deaths. Sacks and Seeman reported that the death rate from leukemia in the United States has risen continuously since 1900, with an accelerated rate of increase since 1920. They pointed out that the increase is not

TABLE II

ALL ACCEPTED CASES OF LEUKEMIA IN EXPOSED SUBJECTS RECORDED BY THE COMMISSION WITH THE INDICATION OF THE METHOD OF CONFIRMATION AVAILABLE

Number	Distance from Hypocenter (meters)	Date of Death	Seen at ABCC	Blood Smear	Bone Marrow	Death Certificate	Hospital Record	Autopsy
1	700	1948	-	-	-	x	-	-
2	900	Living	x	x	-	-	-	-
3	950	Living	x	x	x	-	-	-
4	950	1950	x	x	-	x	-	-
5	950	Living	x	x	x	-	-	-
6	950	1950	-	x	-	x	x	-
7	970	1950	x	x	x	x	-	-
8	1020	1950	x	x	x	x	-	-
9	1040	1949	-	-	-	x	-	-
10	1045	1949	x	x	x	x	-	x
11	1050	1950	-	-	x	-	x	-
12	1050	Living	x	x	x	-	-	-
13	1100	1949	x	x	-	x	-	-
14	1100	1950	x	x	x	x	-	-
15	1130	1950	x	x	x	x	-	-
16	1150	1950	x	x	x	x	-	-
17	1150	Living	x	x	-	-	-	-
18	1150	1948	-	x	-	x	x	-
19	1170	Living	x	x	x	-	-	-
20	1190	Living	x	x	x	-	-	-
21	1220	1948	-	-	-	x	-	-
22	1300	1950	x	x	x	x	-	-
23	1350	Living	x	x	x	-	-	-
24	1350	1949	-	x	-	x	-	x
25	1400	1949	-	x	-	x	x	-
26	1440	1947	-	-	-	x	-	x
27	1450	1948	-	x	-	x	x	-
28	1475	Living	-	x	-	-	x	-
29	1500	1948	x	x	x	x	-	-
30	1520	Living	x	x	x	-	-	-
31	1530	1950	x	x	x	x	-	-
32	1550	1950	-	x	-	x	x	x
33	1600	1948	-	x	-	x	x	x
34	1750	1949	-	-	-	x	-	x
35	1820	1949	-	-	-	x	-	-
36	1830	1948	-	x	-	-	x	-
37	2110	1950	x	x	x	-	-	-
38	2175	1951	-	x	x	-	x	-
39	2400	1951	-	x	-	x	x	-
40	2650	1951	x	x	x	-	-	-
41	2820	1948	-	x	-	x	x	-
42	3050	1950	-	x	x	x	x	x
43	3600	1951	-	x	x	-	x	-
44	3780	1950	-	x	x	x	x	-
45	5350	1951	-	x	-	x	x	-
46	5420	1949	-	x	-	x	x	-
47	5680	1948	-	-	-	x	-	-

due to changes in the age distribution of the population and that improved diagnostic techniques and greater use of medical facilities must be considered in determining the cause for the rising death rate.[4] The lower incidence of leukemia, as reported in Japan, may be due to a relative lack of medical facilities available and the ratio of physicians to a large rural population. The available figures suggest that the incidence in Japan may actually be lower than in the United States.

In the absence of a satisfactory basis for predicting an expected incidence and death ratio it has been considered desirable to compare the incidence and death rate from leukemia in the exposed and non-exposed populations of the cities of Hiroshima and Nagasaki. Further, a comparison of the incidence and death rate from leukemia is made between the population exposed under 2,000 meters and 2,000 meters and over.

TABLE III

OFFICIAL FIGURES ON THE INCIDENCE OF LEUKEMIA IN ALL JAPAN INCLUDING OKINAWA FOR THE YEARS 1935–1940, 1948, 1949* AND THE INCIDENCE IN THE UNITED STATES FOR THE YEAR 1940**

Year	Population	Total Deaths	Leukemic Deaths	Leukemic Deaths per 10^6 Living	Leukemic Deaths per 10^4 Total Deaths
1935	69,254,148	1,161,936	969	14.0	8.3
1936	70,258,200	1,230,278	991	14.1	8.1
1937	71,252,800	1,207,899	930	13.1	7.7
1938	72,222,700	1,259,805	911	12.6	7.2
1939	72,875,800	1,268,760	930	12.8	7.3
1940	73,114,308	1,186,595	939	12.8	7.9
1948	80,200,000	950,610	956	11.9	10.1
1949	82,200,000	945,444	1,120	13.6	11.8
UNITED STATES					
1940	131,669,000	1,417,285	5,135	39	36.2

*The Division of Health and Welfare Statistics, Welfare Minister's Secretariat, Tokyo

**Based on a publication of the United States Department of Commerce-Vital Statistics Rates in the United States 1900-1940 by Linder, F.E. and Grove, R.D.

A total of thirty cases and twenty-four deaths were recorded in Hiroshima as compared with nineteen cases and nineteen deaths in Nagasaki. An analysis by the Chi square and interaction method shows no significant difference between the two cities.

In the total exposed population of the two cities twenty-nine cases and twenty-three deaths are recorded as compared with twenty cases and twenty deaths in the non-exposed population. This is a highly significant increase in the incidence and deaths from leukemia in the exposed population as shown by the Chi square analysis.

In Table v the cases of leukemia and deaths from leukemia for 1948, 1949, and 1950 in the exposed population are presented according to distance from the hypocenter of the atomic explosion. A total of twenty-two cases and eighteen deaths from leukemia were recorded in the population exposed under 2,000 meters as compared with seven cases and five deaths in the population exposed at 2,000 meters or over. A comparison of these data by the Chi square test reveals a highly significant increase in both the incidence and deaths from leukemia in the group exposed at a distance of less than 2,000 meters from the hypocenter.

PRINCIPAL FINDINGS AND THEIR INTERPRETATION

Incidence of Leukemia. Table IV lists the cases with onset of leukemia and deaths from leukemia during the years 1948, 1949 and 1950 in the cities of Hiroshima and Nagasaki.

The data for deaths from leukemia in Table v are graphically represented by Figure 1 in which the per cent of total deaths, occurring in the combined cities, contributed by the various segments of the exposed population is compared with the per cent of the total exposed population it comprises 80 per cent of the total exposed population.

Figure 2 represents the number of deaths from leukemia in subjects at various distances from the hypocenter expressed as deaths per 10^6 living persons in each group. Table vi pre-

TABLE IV

CASES WITH ONSET OF SYMPTOMS OR DEATH FROM LEUKEMIA DURING 1948–1950 INCLUSIVE IN THE POPULATION OF THE CITIES OF HIROSHIMA AND NAGASAKI

	EXPOSED			NON-EXPOSED			TOTAL		
	Population	Cases 1948-1950	Deaths 1948-1950	Population	Cases 1948-1950	Deaths 1948-1950	Population	Cases 1948-1950	Deaths 1948-1950
Hiroshima	98,265	19	13	187,447	11	11	285,712	30	24
Nagasaki	96,962	10	10	144,843	9	9	241,805	19	19
Total	195,227	29	23	332,290	20	20	527,517	49	43

Summary of Comparison

		Number of Cases of Leukemia		Deaths from Leukemia	
Comparison	D/F	X^2	P	X^2	P
Hiroshima vs Nagasaki	1	0.99		0.05	
Exposed vs Unexposed	1	10.34	0.002	5.01	0.03
Interaction	1	3.30		0.47	

TABLE V

CASES OF LEUKEMIA WITH ONSET OF SYMPTOMS OR DEATH FROM LEUKEMIA DURING 1948–1950 INCLUSIVE IN THE CITIES OF HIROSHIMA AND NAGASAKI ACCORDING TO DISTANCE FROM HYPOCENTER

	HIROSHIMA CITY			NAGASAKI CITY			TOTALS (combined areas)		
Distance from Hypocenter (meters)	Exposed Population	Cases of Leukemia	Deaths from Leukemia	Exposed Population	Cases of Leukemia	Deaths from Leukemia	Exposed Population	Cases of Leukemia	Deaths from Leukemia
0- 999	1,400	3	1	671	1	2	2,071	4	3
1000-1499	10,596	8	5	3,227	4	4	13,823	12	9
1500-1999	19,002	4	3	4,361	2	3	23,363	6	6
Under 2000	30,998	15	9	8,259	7	9	39,257	22	18
2000-over	67,267	4	4	88,703	3	1	155,970	7	5
Totals	98,265	19	13	96,962	10	10	195,227	29	23

Comparison of Incidence in Combined Cities of Cases and Deaths from Leukemia Exposed under 2000 Meters vs 2000 Meters or over

	Cases of Leukemia	Deaths from Leukemia
X^2	56.12	48.42
D/F	1	1
P	<.001	<.001

comprised by each segment. The population exposed at distances up to 1,999 meters contributed 78 per cent of all deaths from leukemia although it comprises only 20 per cent of the total exposed population. The population exposed at 2,000 meters or over contributed only 22 per cent of the leukemic deaths although

sents the calculated death rate from leukemia in the exposed populations of the two cities by distance from the hypocenter. The death rate per 10^6 living persons in the population exposed at distances less than 2,000 meters is high as compared with the death rate in the population exposed at 2,000 meters or over.

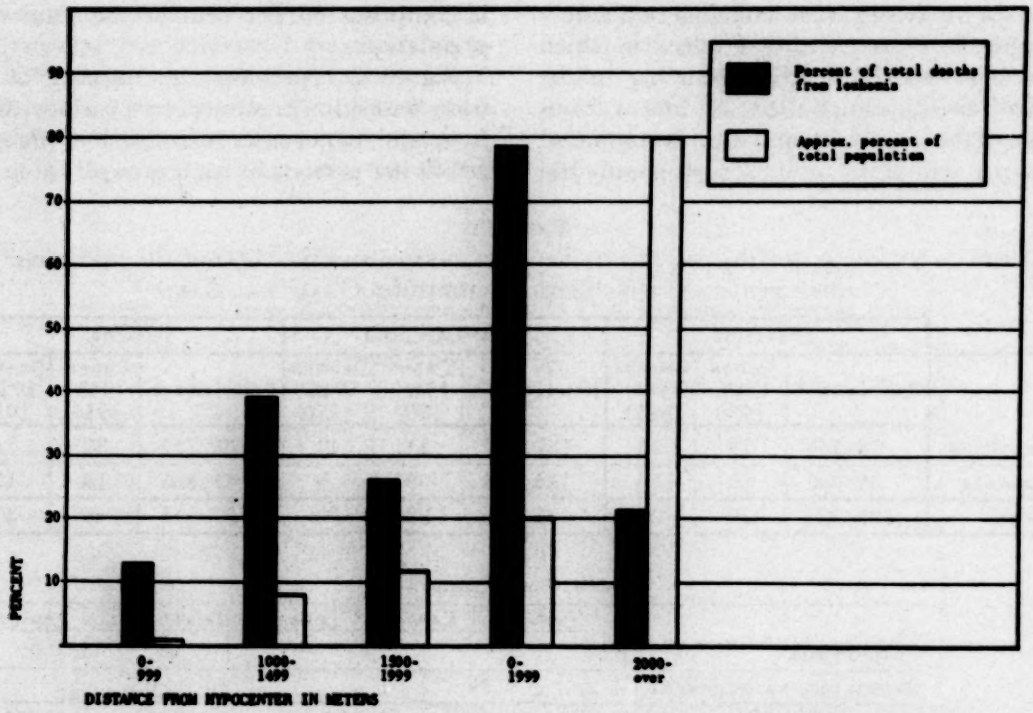

FIG. 1. Comparison of per cent of total deaths from leukemia in the cities of Hiroshima and Nagasaki during 1948, 1949 and 1950 contributed by various segments of the exposed population compared with the per cent of total exposed population comprised by each segment.

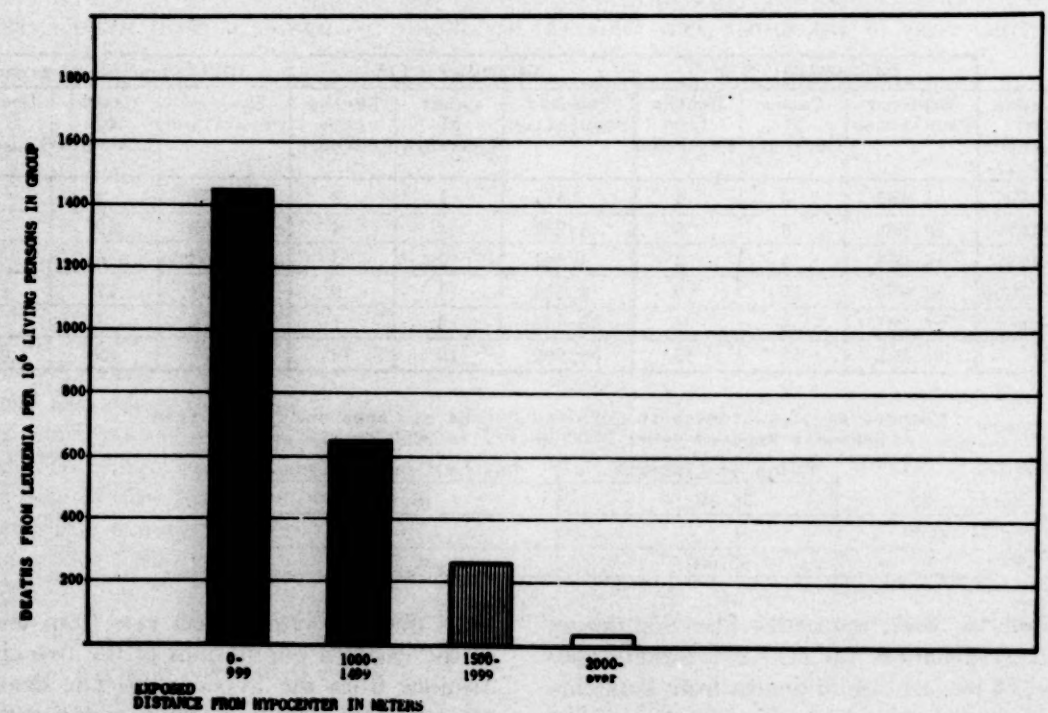

FIG. 2. Number of deaths from leukemia in Hiroshima and Nagasaki cities 1948–1950 occurring in the exposed groups expressed as deaths per 10^6 living persons in that group.

Acute Radiation Symptoms. It should be recognized that due to shielding, and perhaps other factors such as individual resistance, a significant percentage of the survivors exposed to radiation at distances less than 2,000 meters gives no history of acute radiation illness following the exposure.* A positive radiation history in the population exposed beyond 2,000 meters is unusual. It becomes significant in comparing the incidence of leukemia in the exposed populations according to distance from the hypocenter to document actual exposure to radiation injury.

Data are available on forty-seven exposed patients who developed leukemia with reference to symptoms of radiation injury following the bombing. (Fig. 3.) Epilation, purpura and oropharyngeal lesions are reliable signs of acute radiation effect. It has been recognized that fever and vomiting may be non-specific symptoms unrelated to radiation. However, all radiation histories were taken by physicians and the symptoms and signs were evaluated as to the time of appearance, relationship to other portions of the medical history and the accuracy and intelligence of the informant before being recorded. The radiation histories represent signs and symptoms based on the etiologic factor of radiation as well as can be determined.

While the case numbers are small and the incidence of the symptoms varies somewhat from group to group, fever and epilation show a striking progressive diminution of occurrence as distance from the hypocenter increases. Epilation, which occurred in all of the patients with leukemia exposed at distances less than 1,000 meters, was also more severe in this group, being complete in 75 per cent of the cases. The symptoms and signs of radiation injury appear to diminish rapidly in the patients exposed beyond 2,000 meters. Whereas epilation of some degree occurred in 70 per cent of the patients exposed under 2,000 meters, it was not observed in the eleven patients exposed beyond this point. Purpura also was not found in the latter group, and a positive history of the other symptoms listed was found only in a single case.

The presented data in the cases of leukemia exposed at distances less than 2,000 meters documents the actual exposure to radiation injury by a high incidence of symptoms and signs indicative of a severe radiation insult. This is again evidence supporting the concept of radiation exposure as a leukemogenic agent in man.

Age Distribution. In the United States leukemia affects persons in the older age groups, fifty-five years and over, with the greatest frequency and the death rate from leukemia is lowest in the intermediate ages.[4] Forty-six cases of leukemia, developing since 1945, in the exposed population and thirty cases in the non-exposed population of Hiroshima and Nagasaki in whom the age at onset of symptoms could be determined are presented in Figure 4.

In the thirty-six patients exposed at distances less than 2,000 meters approximately 86 per cent (thirty-one patients) had onset of symptoms before the age of forty-five years and in no

TABLE VI

DEATHS OBSERVED FROM LEUKEMIA AND CALCULATED DEATH RATE FROM LEUKEMIA IN THE EXPOSED POPULATIONS OF HIROSHIMA AND NAGASAKI FOR THE YEARS 1948, 1949 AND 1950

Distance from Hypocenter (meters)	Area	Exposed Population	Deaths from Leukemia Observed	Death Rate from Leukemia per 10^6 Living Persons
0-1,999	Hiroshima	30,998	9	290
	Nagasaki	8,259	9	1080
	Total	39,257	18	458
over 2000	Hiroshima	67,267	4	59
	Nagasaki	88,703	1	11
	Total	155,970	5	32
All Distances	Hiroshima	98,265	13	132
	Nagasaki	96,962	10	103
	Total	195,227	23	118

* Atomic Bomb Casualty Commission unpublished data.

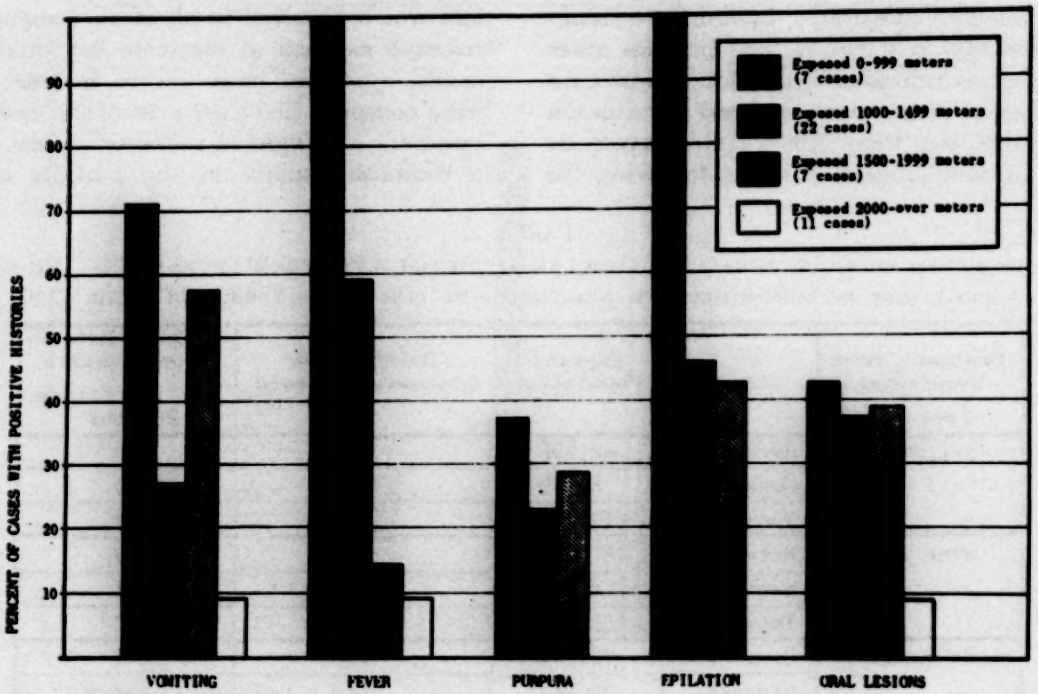

FIG. 3. Incidence of symptoms and signs of radiation injury at time of atomic bomb in forty-seven subjects exposed in Hiroshima or Nagasaki in whom leukemia subsequently developed.

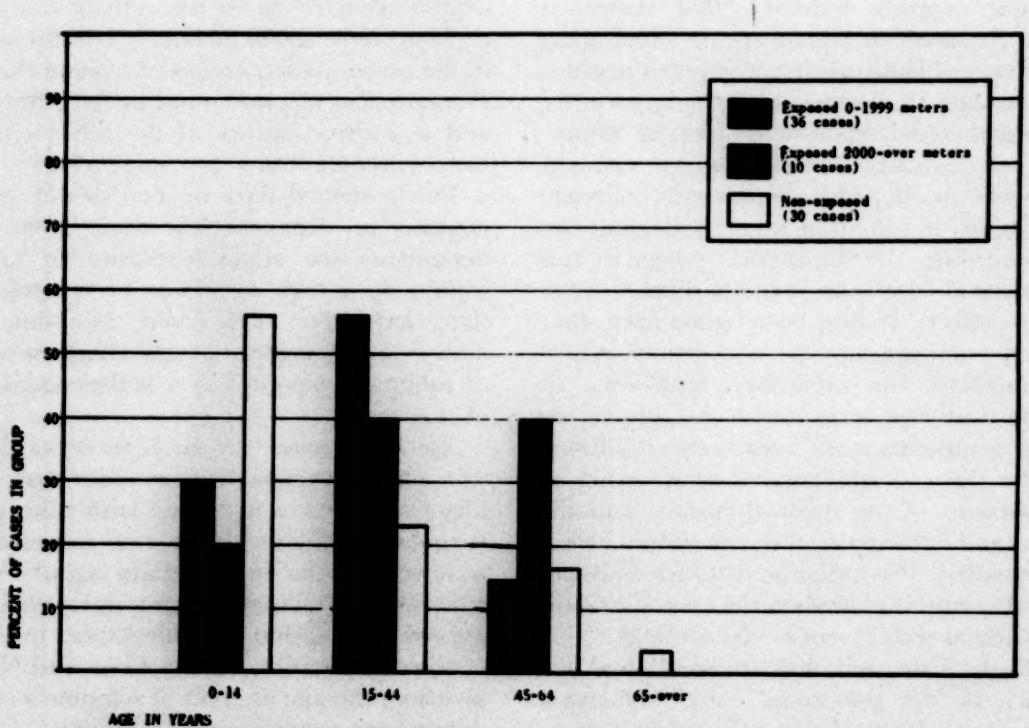

FIG. 4. Age distribution based on age at onset of symptoms in seventy-six cases of leukemia developing in Nagasaki and Hiroshima areas since the atomic bomb.

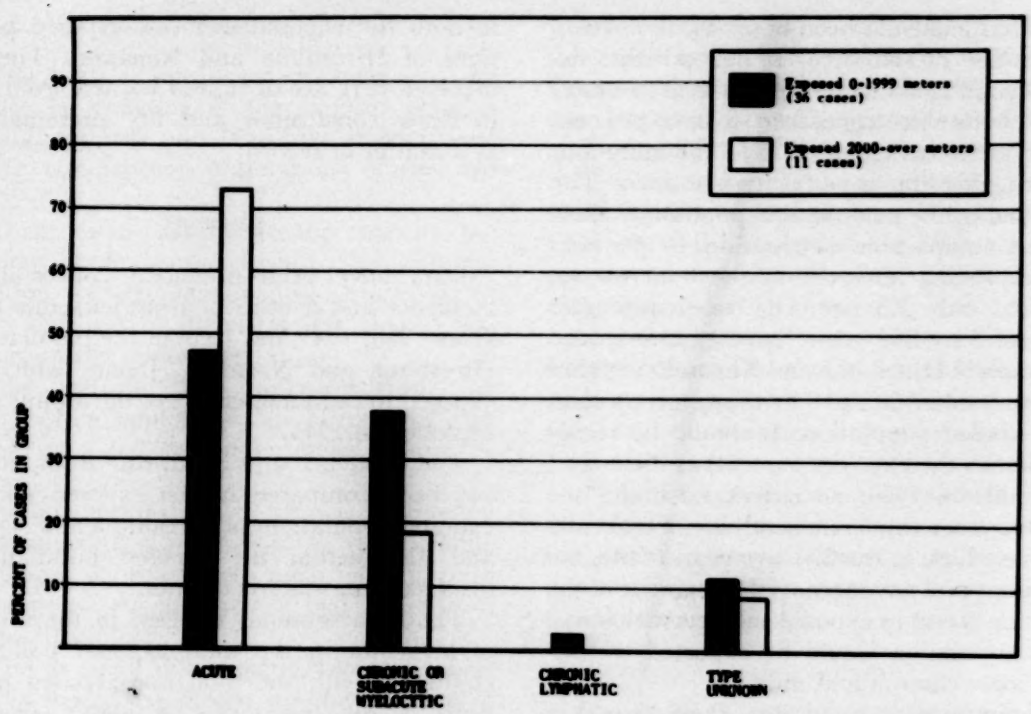

FIG. 5. Distribution by type of leukemia of the forty-seven exposed patients with leukemia in Hiroshima and Nagasaki areas since the atomic bomb.

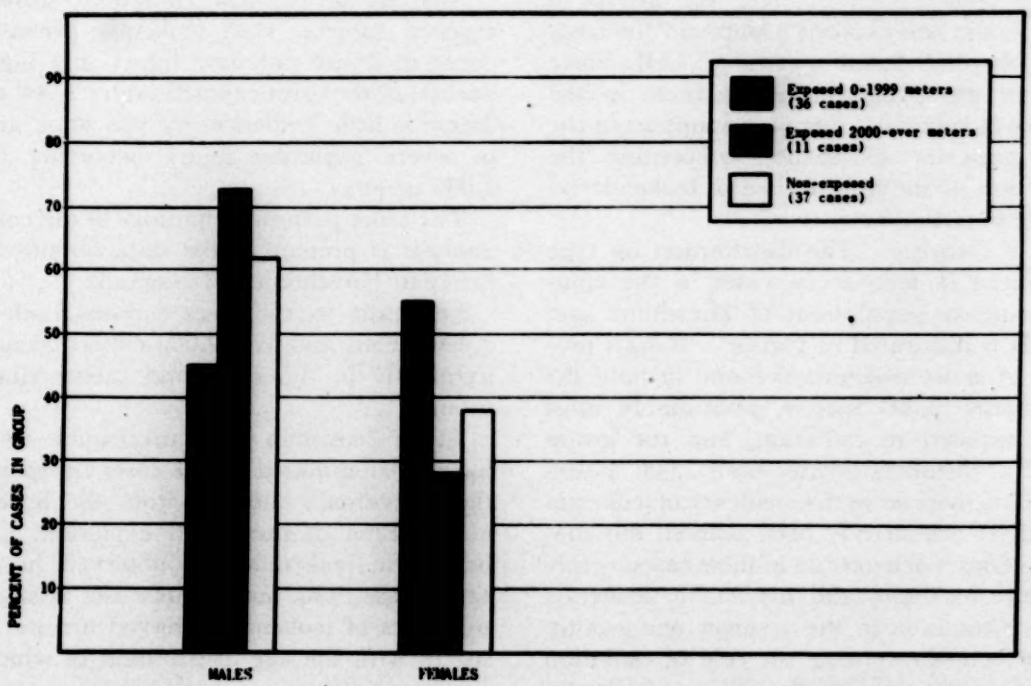

FIG. 6. Distribution by sex of the cases of leukemia occurring in Hiroshima and Nagasaki areas since the atomic bomb.

patients has leukemia been observed to develop after the age of sixty-five. In ten patients exposed beyond 2,000 meters 60 per cent (6 cases) occurred before the age of forty-five, 40 per cent (4 cases) between ages forty-five and sixty-four and none after the age of sixty-five years. The cases found in the non-exposed population show a roughly comparable distribution, 80 per cent (24 cases) being observed in the 0 to 44 age group and only 3.3 per cent (one case) after the age of sixty-five years. In the non-exposed populations of Hiroshima and Nagasaki a higher incidence is noted in the 0 to 14 age group than in the exposed population. It should be recognized that in the exposed population there was, for example in 1950, no individual under the age of five years. Since the incidence of leukemia is relatively high in the first two years of life, the advancing years have removed a segment of the 0 to 14 age group in exposed subjects which in a normal population would be expected to contribute some cases of leukemia.

It is tempting to read into these figures a possible shift in the age of onset of leukemia to a younger than average group and to speculate as to the possible role of radiation as a causative agent. However, it appears that a similar incidence of leukemia in younger age groups is present in the non-exposed group and the cases exposed beyond 2,000 meters as well. Since this latter group showed no increase in the incidence of leukemia, there is no support in the present data for speculation concerning the acceleration of the appearance of leukemia by exposure to radiation.

Type of Leukemia. The distribution by type of leukemia in forty-seven cases in the combined exposed populations of Hiroshima and Nagasaki is illustrated in Figure 5. A high proportion of acute leukemia is found in both the group under 2,000 meters, presumably most heavily exposed to radiation, and the group exposed at distances greater than 2,000 meters in which no increase in the incidence of leukemia is found. It has already been pointed out that the age of onset of leukemia in these cases is early and acute leukemia and myelocytic leukemia are more common in the younger age groups. No statement concerning the role of radiation in the determination of the type of leukemia which develops is warranted on the basis of the present data.

Sex Distribution. Figure 6 indicates the distribution by sex of all known cases of leukemia in both the exposed and non-exposed populations of Hiroshima and Nagasaki. The cases involved here are obviously too few from which to draw conclusions and are presented only as a matter of record.

SUMMARY

Data have been presented concerning the incidence and death rate from leukemia for the years 1948, 1949 and 1950 in the populations of Hiroshima and Nagasaki, Japan, which were exposed to radiation effects of the atomic bombs exploded in 1945.

The incidence and death rate from leukemia has been compared in the exposed and non-exposed populations of Hiroshima and Nagasaki and also within the exposed population by distance from the hypocenter.

The data show an increase in the incidence of leukemia in the total exposed populations compared with the total non-exposed populations of the two cities. A highly significant increased incidence of leukemia is found in the subjects exposed to the radiation at distances of less than 2,000 meters as compared with those exposed beyond 2,000 meters.

Analysis of medical radiation histories in exposed subjects with leukemia presents evidence of severe radiation injury in a high proportion of the cases exposed under 2,000 meters. There is little evidence, by this same analysis, of severe radiation injury occurring beyond 2,000 meters.

The same pattern of findings of the collective analysis is present in the data obtained separately in Hiroshima and Nagasaki.

Leukemia in the cases exposed both under 2,000 meters and over 2,000 meters occurs most frequently in the early and intermediate age groups.

Acute leukemia and myelocytic leukemia have predominated in all cases irrespective of the individual's distance from the hypocenter at the time of the bomb explosion. Chronic lymphatic leukemia was observed in only a single case. The number of cases is small and the types of leukemia observed are not inconsistent with the age distribution in which they occurred.

Comparative differences in the sex distribution in the cases of leukemia are slight and the total numbers too small to warrant any conclusions.

CONCLUSIONS

1. There is a significant increase in the incidence of leukemia in the exposed populations of Hiroshima and Nagasaki as compared with the non-exposed populations of the two cities.

2. There is a significant increase in the incidence of leukemia within the exposed population of Hiroshima and Nagasaki in subjects exposed at distances less than 2,000 meters from the hypocenter.

3. The concept that radiation from the atomic bomb explosions in Hiroshima and Nagasaki is a leukemogenic agent in man is supported.

4. No conclusions can be drawn concerning the relationship of radiation to the possible acceleration of the appearance of leukemia or the type of leukemia which may develop in the exposed subjects.

5. The present study should continue and emphasis should be placed on the position of these data relative to a curve of increased incidence of leukemia in the exposed population under 2,000 meters, and the presence or absence of an increased incidence of leukemia occurring at a later date in the populations exposed to radiation beyond 2,000 meters.

Acknowledgment: The material presented in this report is from a survey by the Atomic Bomb Casualty Commission with the cooperation of the Japanese physicians, medical institutions and municipal health agencies. Doctor Yoshimichi Yamasowa was responsible for some of the early aspects of the survey. The medical department of the Commission in Hiroshima under the direction of Dr. Paul G. Fillmore and in Nagasaki under Dr. James N. Yamazaki have contributed greatly. The advice of Dr. John S. Lawrence and Dr. William N. Valentine, consultants in medicine to the Atomic Bomb Casualty Commission, was most helpful. Dr. Valentine's assistance in the final collection and analysis of the material was invaluable. Finally, this report could not have been written without the generous cooperation and assistance of all departments of the Atomic Bomb Casualty Commission.

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Review

Renal Medullary Necrosis*

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NECROTIC lesions of the kidney confined to the distal parts of the pyramids and not resulting from purulent destruction, pressure atrophy, obstruction of a larger blood vessel or intoxication have been known for some time. Their pathogenesis has been the subject of much discussion, formerly in Europe, more recently in the United States also. The present summary of available clinical, pathologic and experimental data has been prepared in the hope that it will provide a useful basis for fuller elucidation of the problem in the future.

Incidence. Pyelonephritis has been found on the autopsy table to occur in 18 to 22 per cent[1–3] of diabetic patients but in only 3.5 to 4 per cent[4,5] of general postmortem material. Of 859 diabetic patients autopsied at Los Angeles County Hospital,[6] acute pyelonephritis was considered in 107 to be the cause of death or contributed thereto; twenty-nine patients in this group (27.1 per cent) showed evidence of "papillary or pyramidal necrosis." Of the sixty patients with acute pyelonephritis included in another autopsy study on 307 patients with diabetes[3] sixteen showed "necrotizing papillitis."† Hence the incidence of this lesion in diabetic patients was 3.4 to 5.2 per cent and in diabetic patients suffering from pyelonephritis about 27 per cent. In contrast it was found among 9,600 necropsied non-diabetic patients seven times[7] and among 31,141 similar cases[8] twenty-one times, an incidence of about 0.07 per cent; these twenty-one patients also exhibited the finding of acute pyelonephritis of which there was a total of 1,023 instances. In other words of every 100 pyelonephritic patients without diabetes two had papillary necrosis.

In the available literature 160 cases of papillary necrosis‡ were found to have been reported in some detail (Table 1),§ the vast majority after 1937 when the frequent association of the lesion with diabetes mellitus was first emphasized by Froboese and Günther,[15,16] By 1948 the latter authors had observed twenty-nine cases of which only four were without diabetes.[6] The predominance of diabetes in the tabulated cases (96 of 160) is in keeping with the findings of the survey studies mentioned previously. Obstruction of the urinary tract was found in at least forty-eight non-diabetic patients and fifteen with diabetes. The actual incidence of this feature may have been higher since it is not referred to in some case reports. The same is true of the frequency of unilateral involvement which was apparently present in more than one-third of the cases in both the diabetic and non-diabetic group. In general medullary necrosis, when associated with diabetes, was encountered far more often without obstruction than with (about five to one), while in non-diabetic patients that proportion was reversed (one to three). Only four of ninety-one diabetic patients listed‖ were less than forty years of age; fifty-two were between fifty and seventy, and seventeen were more than seventy years old. Of sixty-two non-diabetic patients,‖ eleven were below forty years of age; twenty-two ranged between fifty and seventy, and twenty had passed their seventh decade. There were sixty-six women among the ninety-one diabetic patients, but men predominated among the non-diabetic patients (forty-five of sixty-two), a reflection of the frequent occurrence of urinary obstruction in elderly men.

In view of the reported autopsy incidence

† This and similar terms are rejected by Günther[6] who, for plausible reasons, upholds the name papillary or medullary necrosis.

‡ Medullary and papillary necrosis, as clinical terms, are used interchangeably in this paper, the papilla being a part of the renal medulla.

§ The diagnoses in the early cases listed may not be too reliable; in particular the two non-diabetic cases of Turner's[9] and all of Stoudensky's cases[10] have been questioned by Günther.[16]

‖ Data concerning age and sex were not included in some of Günther's[16] and Alken's[20] case reports.

* From the Communicable Disease Center, Public Health Service, Federal Security Agency, Atlanta, Ga.

this disease may be expected to be the cause of death in a considerable proportion of the diabetic population in this country unless it will be prevented or treated more successfully than in the past. Prevention obviously depends upon early recognition and prompt, effective treatment of its usual forerunner, pyelonephritis.[5–7,23,25,30,32] Even with the growing number of specific antibacterial drugs available, the frequently insidious symptomatology of obstructive uropathy and of kidney infection, particularly in diabetic patients,[16,25,37,38] may continue to be a source of delay and frustration. This fact is illustrated by the difference in the reported incidence of pyelonephritis as found among diabetic patients[39] and as determined in necropsied diabetic patients,[1–3] that is, 6 per cent and 18 to 22 per cent, respectively.

TABLE I
160 CASES OF MEDULLARY NECROSIS OF THE KIDNEY REPORTED IN LITERATURE

Year of Publication	Author	Diabetes			
		Present		Absent	
		Urinary Obstruction			
		Present	Absent	Present	Absent
1877	Friedreich[6]			1	
1888	Turner[9]		1(1)		2(1)
1899	Stoudensky[10]			6(5)	
1926	Artusi[11]				1
1931	Schömer[12]		1		2
1934	Grauhan[13]			2(2)	
1934	Foulon *[14]				1(1)
1937	Froboese[15]		9(7)		1
1937	Günther[16]	2(2)†	6(1)	2	
1937	Praetorius[17]			1(1)	
1937	Sheehan[18]			1	
1938	Junker[19]		1(1)		
1938	Alken[20]		4(4)		
1938	Schneider[21]				1(1)
1941	Mellgren *[22]			1	1
1939	Olsson[23]		1(1)		
1942	Overzier[24]			1(1)	
1942	Harrison *[25]		3(1)		
1944	Davson *[26]				1
1945	Eskelund[27]				1
1946	Robbins *[7]		9(2)	6	1
1947	Edmondson *[5]	8(4)	21(4)	20(9)	1(1)
1947	Pellegrin[28]		1		
1947	Dahlmann[29]		1(1)		1
1948	Richfield[30]		1		
1948	Stevens *[31]		1		
1948	Moore[32]		1		
1949	Welch *[33]		1		
1949	Robbins *[34]	2(1)	6(3)	5	1
1950	Gaustad *[35]	3(2)	2(2)	1(1)	
1950	Muirhead *[36]		1	1(1)	1
Total		15(9)	81(28)	48(20)	16(4)
		96(37)		64(24)	

* Signifies co-authors omitted from table.

† Numbers in parentheses represent number of cases with unilateral involvement.

Clinical Diagnosis and Treatment. Success of treatment of a patient with papillary necrosis may conceivably depend on its early detection. The symptoms, however, of the primary disease usually completely mask the presence of the necrotic lesion.[5–7,26] Only sporadic cases are reported to have been diagnosed clinically, mostly on the strength of the roentgen examination.[5,6,15,20,22,25,33] (A number of cases labeled "necrotising papillitis" on the basis of clinical and x-ray examinations were not verified anatomically.)[21,40,41] Among frequent clinical findings cited are flank pain, dysuria, urinary urgency and frequency; proteinuria, pyuria, bacteriuria, microscopic to massive hematuria; oliguria and anuria; azotemia (without appreciable hypertension); chills, fever, nausea and vomiting, prostration, stupor and coma (with or without diabetic acidosis). The disease may set in as an acute fulminating septicemia with rapidly advancing uremia and death within a few days;[5,7,28,31,32–34] it may appear as a sudden uncontrollable exacerbation of a protracted pyelonephritis[7] or, in some instances, the course may be slowly progressive over a period of weeks or months toward the usual (but not invariable) fatal termination.[22,23,26,35] According to Edmondson and his associates[5] criteria for diagnosis in diabetic patients include hematuria, renal colic, coma and a sudden increase in the severity of symptoms of known pyelonephritis. However, in non-diabetic patients with obstructive uropathy these authors found, even in retrospect, no clue to an antemortem diagnosis of associated papillary necrosis. Robbins and Angrist[34] postulated that the lesion should be suspected in any severe acute infection of the urinary tract in diabetic patients and in all non-diabetic patients with urinary obstruction, especially if oliguria and a rising blood nonprotein nitrogen are present. Günther[6] stressed the usual clinical picture of "urosepsis," a com-

bination of renal or generalized infection with renal insufficiency, and warned of a need for awareness of the possibility of a change from diabetic coma to uremic coma. Mellgren and Redell[22] believed that the frequent combination of three findings should quite readily delimit the clinical picture of the disease from "purulent pyelitis or pyelonephritis": hematuria, attacks of renal colic and, most important, the x-ray.

Necrotic tissue sequesters—a conceivable cause of colic or ureteral obstruction—were in rare instances excreted or obtained through cystoscopy and allowed a histologic diagnosis.[6,15,20] More often unilateral nephrectomy made possible recognition of the lesion *in vivo*.[5–7,13,16,17,19–21,29,33] In some instances it probably contributed to the patient's survival[5,7,13,17,19,21,25,29,34] even though freedom from necrosis of the other kidney was not always ascertained.[5,7,23,25] Spontaneous recovery was observed in occasional cases[5,6,20,24,34] and, by some authors, assumed to be a definite possibility.[21,40,41] Consequently, and because of the possible injurious effect of nephrectomy on the function of the remaining kidney, there seems to be no final agreement as to whether an apparently unilateral necrosis constitutes an urgent indication for operative removal of the organ or whether the patient should be treated conservatively. According to Günther[6] nephrectomy is indicated only if (1) sequestrated papillae seem to entertain infection as suggested by pain, pyuria or bleeding, or (2) if urinary obstruction is present. American authors are more inclined toward radical surgery[5,7,23,34] which may occasionally be confined to nephrostomy.[42]

Roentgenologic Findings. Obviously, of all types of clinical examination the retrograde pyelogram is most apt to demonstrate destruction of renal papillae or medulla. Yet this may not be possible in the early stage when the silhouette of the calyces may be unaffected or show only changes consistent with pyelonephritis.[5,6] Demarcation and separation of a papilla may result in its arcade-like delineation against the medulla ("sling"[6] or "ring"[20] shadow), in disappearance of the papillary tip with consequent cavity formation[6,20,22] or in a concrement-like defect within the opaque medium outlining the renal pelvis.[6,23] When the necrotic process originates in the more proximal parts of the pyramid,[6,7] it may not be visualized until breakdown of the surface of the papilla permits the contrast medium to penetrate into the medullary cavity created by collapse or resorption of necrotic tissue.[6] The changes in the roentgen picture may simulate those caused by pyelovenous backflow, by pyelonephritis without necrosis, by tuberculosis, tumor or calculus.[5,6,20,23,40,41]

Pathologic Anatomy. In the vast majority of published cases the diagnosis was not made until autopsy. The gross lesion is readily recognized by the pale color of the involved papillae or entire pyramids, standing out in sharp contrast against the surrounding parenchyma. This border is often made more prominent by a zone of demarcation along which separation may occur, resulting in the finding of necrotic particles along the lower urinary tract; they may act as nuclei for renal stones, or be excreted,[6,8,12,15,22,29,43] and may be mistaken for tumor particles.[15] Microscopically, there is coagulation necrosis with pycnosis or absence of cell nuclei and loss of cellular detail but persistence of the general architecture. The cortex and columns of Bertini usually show advanced pyelonephritis, often with abscess formation but no necrosis. The area of demarcation contains numerous inflammatory cells and, toward viable tissue, marked congestion. Some authors[5,28,35] found large mononuclear cells to be prevalent in diabetic cases. Thrombosed blood vessels in and surrounding the necrotic area have frequently been encountered and implicated in the development of the necrosis,[5–7,15,25,31,32,34,35] much as peripheral vascular disease causes gangrene of limbs.

Throughout the parenchyma, bacteria were usually found in large numbers.* Escherichia coli and staphylococci occurred most often but streptococci, Klebsiella pneumoniae, Pseudomonas aeruginosa, proteus and leptothrix were also reported present.[5–7,15,16,18,22,34,35] In two instances, Mycobacterium tuberculosis[5,10] and in one, actinomyces[5] were found. The morphologic findings do not appear to depend on the type of infecting organism but vary chiefly in accordance with the stage of the lesion.[7] After sequestration or resorption of necrotic material healing through fibrosis may occur as evidenced by several anatomic studies[5,20,24,34] and suggested by clinical observations.[21,40,41]

Pathogenesis. The sequence of events as reconstructed by Robbins and Angrist[34] and concurred in by most recent observers[5,6,25,28,31,32]

* With the exception of a case of papillary necrosis in a diabetic patient reported by Schmorl in 1907 who claimed complete absence of bacteria.[6]

is an initial, rapid, complete infarct-like necrosis of the affected region precipitated by the infectious process, followed with an inflammatory reaction. The former authors discounted the theory that coalescence of small abscesses at the base of the pyramid initiated the distal ischemic infarction.[7]

With far advanced lesions only presenting themselves for direct inspection at surgical operation or postmortem examination, animal experiments could be expected to aid in speculations about the pathogenesis. Some investigators utilized one or both of the factors most prominent in the human disease, namely, urinary obstruction and infection. Others, without introducing such complicating manipulations, produced necrosis in the renal medulla through dietary or chemical means.

Robbins and his group[3,7] observed the "exact analogue" of the human lesion, as found commonly in non-diabetic patients, after ligation of one ureter in the rabbit and intravenous injection of pyogenic organisms; the non-obstructed kidney showed no significant involvement. Papillary necrosis had previously been observed in similar experiments, namely, after simple ligation of a ureter,[10] combined ureteral obstruction and retrograde injection of bacterial cultures[44] and intravenous injection of staphylococci in the presence of intact ureters.[48] Ureteral ligation as applied by Muirhead and his coworkers[36] either bilaterally or unilaterally and combined with contralateral nephrectomy, resulted in hydronephrosis and necrosis of renal papillae in eighteen of twenty-eight dogs, often associated with bacterial infection.

In rats ureteral obstruction failed to produce papillary necrosis even when combined with pancreatectomy.[5] Rats maintained on a fat-free diet were found to develop circumscript necroses of the papillae combined with calcification.[46] However, the most prominent and constant lesion was calcium deposition in the cortical tubular epithelium and in casts; the pyramids were not directly involved except for their tips. A parallelism between this and the human lesion has been suggested[34] but is far from being manifest.

Certain chemicals, such as tetrahydroquinoline[47] and vinylamine,[48] have for some time been known to cause necrosis of the renal medulla in various species. A recent study of vinylamine intoxication in rabbits disclosed that the necrosis was preceded by a brief stage of excessive medullary hyperemia, coupled with cortical ischemia,[49] a picture reminiscent of the Trueta shunt.[50] In the few rabbits which survived the necrotic stage there was evidence of fibrotic repair. Because of the fundamental difference between cortical and medullary response in vinylamine poisoning, the medullary necrosis (stage II) was not likely to be the direct result of a nephrotoxic action. Rather, it was attributed largely to acute tissue anoxia resulting from vasoparalysis, congestion and multiple thrombus formation. The characteristic lesion was ischemic coagulation necrosis which began near the papilla and often extended up to the corticomedullary junction; it was usually demarcated by engorged and thrombotic blood vessels and often also by a seam of segmented leukocytes. The necrosis involved both Henle's loops and collecting tubules, interstitium and blood vessels but allowed recognition of the general architecture. Absence of papillary tips indicated sequestration which, in one chronic case, resulted in stone formation and intermittent hydronephrosis. The similarity of morphologic features in this and the human lesion is striking. Functionally, the experimental lesion differed in that it approximated a theoretic lower nephron syndrome: a combination of hyposthenuria and polyuria without proteinuria and hematuria. Evidently the urinary findings in the human disease which, as listed previously, include oliguria, proteinuria and hematuria reflect the usual diffuse extension of the underlying severe infectious process throughout both medulla and cortex. Azotemia —probably, in part, due to back diffusion—is common to both the natural and artificial lesions.

The available data do not permit any final decision as to the various causes of human papillary necrosis which have been proposed by different authors, for example, natural inferiority of the medullary blood supply as compared with the rest of the kidney;[5,7,10,11,16,28,34,35] obliterative vascular disease aggravating the former;[5,9,14,32,35] compression of pyramidal blood vessels and tissue by increased intrapelvic pressure or inflammatory edema[7,8,10,11,13,18,34,36] or by paramyloid,[22] and similar injury due to retrograde pyelography;[14,27] the diabetic state and acidosis;[5,26,34] direct destructive action of bacteria and toxins on tissue;[5,6,16,22] and others. Evaluation of their significance, singly or in combination, is beyond the scope of the evidence at hand. Günther,[6] vigorously rejecting a

"mechanical" genesis (compression of tissue), stressed the infarct-like picture found even in (human and experimental) cases of medullary necrosis classified as toxic* or bacterial. The observations on vinylamine poisoning tend to emphasize vascular trauma and occlusion as an important etiologic factor capable of producing medullary necrosis even in the absence of urinary obstruction, infection or overt disturbance of either carbohydrate or fat metabolism. The high prevalence of human papillary necrosis in diabetic patients and in patients with obstructive uropathy may be explained by the well-known action of the diabetic state and of urinary stasis in favoring the growth and spread of micro-organisms which, in turn, will cause the vascular injury.

SUMMARY AND CONCLUSIONS

Renal medullary necrosis is a serious and often fatal complication of pyelonephritis and is therefore most frequently encountered in patients suffering from diabetes, or urinary obstruction or both. While there are no specific clinical criteria known to distinguish this secondary lesion from the underlying disease, it should be suspected in any case of progressive renal insufficiency when combined with evidence of urinary or systemic bacterial infection. A positive diagnosis *in vivo* can be made through examination of excreted tissue particles or of operative specimens and at times by means of retrograde urography.

In regard to the development of necrosis studies of both human and experimental lesions indicate that occlusion of multiple small blood vessels, precipitated by the toxic-infectious process, plays a major role.

Prevention should be sought in the early detection and elimination of urinary infection and obstruction, particularly in the diabetic patient. Therapeutic considerations may include nephrectomy or nephrostomy, in addition to specific antibacterial medication.

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* This refers to the alleged finding of human papillary necrosis following overdosage of hypnotics,[51] to experimental vinylamine poisoning, and to necrosis of both renal medulla and cortex caused in rabbits by injection of diphtheria toxin.

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Seminars on Gastrointestinal Physiology

Motility of the Alimentary Canal in Man*

Review of Recent Studies

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In comparison to some other fields of medicine, knowledge of gastrointestinal motor function and dysfunction has been accumulated slowly. Except for a few years after the introduction of roentgenologic methods, few periods of sudden advancement have occurred, such as noted in endocrinology, for example, when a new hormone has been isolated and made available. The stimulating impact of new tools for research has not often been felt in the study of motility of the alimentary canal, and criticism of old procedures, such as the use of balloon technics, may often have inhibited the initiation of research. Important knowledge has been accumulated nevertheless. Much of it is of such long-standing that it is now built permanently into current medical and physiologic concepts. Recently, the introduction of devices to record direct pressure and the reexamination, modification and reuse of old technics, together with efforts for quantitative measurement of the activity of the bowel, have brought to light new facts and offer hope for a period of accelerated progress.

METHODS FOR THE STUDY OF THE MOTILITY OF THE ALIMENTARY CANAL IN MAN

Roentgenologic Technics. Roentgenologic procedures are employed most frequently for study of motor activity of the esophagus and gastrointestinal tract in man because of their dual usefulness. They offer not only a means of recognizing disordered motility but afford also a means for detection of pathologic changes. The shortcomings of these methods become most apparent in studies of the mechanisms of bowel action. The necessity of comparatively brief periods of visualization, the reliance on observation only, the lack of a continuous and permanent record from which quantitative measurements can be made and the interference with visualization of one portion of the bowel by opaque medium elsewhere often seriously limit the usefulness of roentgenologic technics. Still, these methods are unsurpassed when a brief observation will suffice and they are essential when exact localization of a balloon or other unit for measuring motility is required.

Balloon Technics. Balloons were first used about sixty-five years ago to record the motility in man by introducing them through a fistula into the stomach.[1,2] It is likely that they will continue to be used for many years because they afford such a simple and inexpensive method of detecting motor activity in the alimentary canal. Also, the records they provide are sufficient for many research and clinical purposes.

Some valid objections exist to the use of balloon systems in studies of motility, particularly if they are employed as a means of determining intraluminal pressures.[3,4] Many of these undesirable features may be avoided by adapting the dimensions of the system to the requirements of the study and by selecting an appropriate recording manometer.

Records obtained from large balloons may sometimes represent the mean of changes occurring in two or more regions of the bowel overlying the balloon; contraction over one portion of the balloon may be neutralized by relaxation over another.[5] This likelihood is greatly reduced if small balloons of the dimensions of a functional segment of bowel, 5 cm. in length or less, are used.[6,7]

It is often stated that the presence of a balloon

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in the gastrointestinal tract stimulates motor activity. Simple contact of the surface of the balloon with the mucosa may conceivably be stimulating. If this does occur, large balloons contacting an extensive mucosal surface would be most likely to offend. Stimulation will result, however, no matter what the size of the balloon if it is inflated sufficiently to distend the walls of the viscus in which it lies. This may be done purposely to induce motor activity;[8] it may be avoided or minimized by the use of small balloons that are not filled sufficiently to stretch the walls of the alimentary canal. The latter situation seems to have been accomplished in the system used routinely in this laboratory,[9] for the balloons, which are small (3 by 5 cm.) and are filled slowly at a pressure of 15 cm. of water, may rest in the bowel for long periods without inducing activity. Also, when balloon and pressure records are made simultaneously in different portions of the bowel, filling the balloon in the prescribed fashion has never initiated motility or altered intraluminal pressure.

When a balloon is stretched within the alimentary canal, the elasticity of the wall of the balloon makes some contribution to the pressure recorded. To minimize this likelihood, no more than 20 to 25 ml. of liquid is placed in a balloon that will hold 35 to 40 ml. before its wall is stretched.[9]

The manometer used to record the changes of pressure within the balloon is an important component of the system. If the system is such that the balloon is easily compressed, the balloon may be emptied before the maximal pressure produced by a contraction is recorded. If the system is too rigid and the balloon is filled with liquid, the balloon may be transformed into a hard, incompressible object resembling a golf ball, the presence of which may act as a stimulus to the bowel or may result in the development of excessive pressures when the bowel contracts.

Thus the volume of gas or liquid displaced from the balloon at different pressures becomes an important consideration. Ideally, the volume of liquid displaced from the balloon at a given pressure should be the same as that which would be moved by the contraction of a segment of the bowel which produced exactly that same pressure. This volume-pressure relationship or coefficient of the stomach or bowel is not known. We have found, however, that a balloon recording system that has a volume-pressure coefficient of approximately 0.13 apparently resembles conditions in the bowel. The objective in setting up such a system has been to mimic conditions in the bowel so that when the gut contracts it will displace the liquid from the balloon into the recording system in precisely the same fashion that it would displace its contents into an adjoining segment. Unfortunately, such systems usually involve the movement of water from the balloon into a cushion of air inserted in the recording system and the volume of liquid that must be moved is usually sufficient to render the system sluggish. In the apparatus currently used in our laboratory, changes in pressure applied periodically to the balloon are followed with fidelity only when the frequency of such changes is 5 to 8 per minute or less.[9,10] (Fig. 1.) If changes in pressure occurring more rapidly than this are to be accurately registered, some other system must be employed.

Technics for Recording Pressure. Changes of pressure within the lumen of the alimentary tract may be used as a measure of its motor activity. Systems utilizing balloons, such as those just described, may or may not accurately measure intraluminal pressures. At present, when the objective of a study is the determination of pressures within the alimentary canal, methods other than balloons should be sought. Open-tipped tubes or catheters, capsules sensitive to pressure or transducers on the ends of various tubes or catheters may be selected.

To be satisfactory for use in man, systems must record with fidelity changes in pressure of 50 cm. or more of water occurring at the fastest rates of rhythmic contractions present in the human gastrointestinal tract. The most rapid cyclic variations of pressure produced by contractions in the bowel that we have observed in human beings have had a frequency of about 12 per minute or 1 cycle per five seconds. Some components of these changes in pressure, such as the rapid upshoot or rapid decline, may require systems capable of following changes in pressure occurring considerably more rapidly. Also, more rapid changes may occur in intraluminal pressure than those we have observed. Therefore, fidelity in the region of 1 cycle per five seconds may be insufficient for accurate recording of all intraluminal changes in pressure in man. Fortunately, systems are available that record large changes in pressure with great fidelity even when they occur rhythmically at frequencies far in excess of 1 per second. (Fig.

1.) These systems employ open-tipped tubes or catheters filled with water or air and connected outside the body to rigid optical manometers,[11,12] to electric strain gauges[13] or to tiny electric pressure-detecting units or transducers inserted into the ends of catheters or tubes that can be placed in the alimentary canal.[10,14–17] These transducers change variations of pressure into variations of electric current and these, when suitably amplified and recorded, give an accurate determination of pressures at the transducer. (Fig. 1.) If open-tipped catheters or tubes are used, they must be kept open by continuous or periodic flushing.

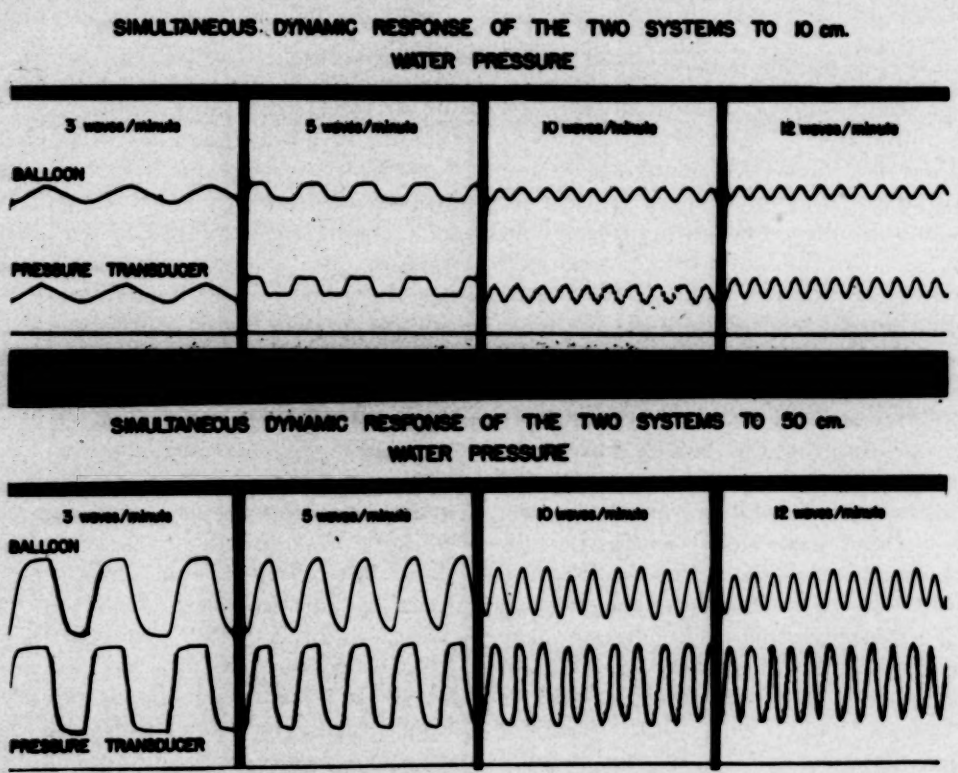

FIG. 1. Comparison of responses of two recording systems to changes in pressure and frequency. Note how the response of the sluggish balloon system ceases to be accurate when changes in pressure occur at rates of 10 or more per minute and how the pressure transducer follows the changes faithfully throughout.

Few comparisons have been made in human beings of the patterns of motility obtained by means of balloons and by means of devices that directly record the intraluminal pressure. In one such study a good correlation was noted between the two methods when pressures in the stomach were recorded.[18] In another study the pressure-detecting device recorded pressure only once every two seconds and considerable discrepancy was noted between records obtained by balloons and those obtained by the device.[19] Much closer correlation between these two methods was noted in the most recent study, in which balloons of the dimensions already mentioned were employed and the electric pressure transducer was used to record the intraluminal pressure continuously.[10,20] More studies of this nature will be needed before the precise features of motor activity that each method determines can be accurately delineated. When this has been accomplished, the investigator will select with assurance the device or combination of devices most likely to give exact quantitative answers to the problem he is attempting to resolve.

CLASSIFICATION OF WAVE PATTERNS SEEN IN MOTILITY RECORDS

When large balloons are used to record motility, the patterns of waves obtained may be so complex that interpretation is exceedingly difficult. For example, simultaneous activity over two or more portions of the balloon will be additive if the motor action is identical but may cancel one another if the action is in opposite directions; if the action is similar but out of

phase, a confusing complex may be recorded.[5] Detection of the motor activity in a small portion or one segment of the alimentary canal simplifies the records obtained and thus aids in their detailed analysis.[6,9] Such pinpoint detection may be accomplished by the use of comparatively small balloons or pressure-pickup units. The use of a number of such balloons or units arranged in tandem allows an estimate of the degree to which the motor activity in one segment is transmitted to or co-ordinated with the activity of its neighbor.[6]

Waves seen in records of motility may be described by their rhythmic characteristics, for example, the "twenty second rhythm" of the antrum,[21] by their size ("S" for small waves, "L" for large waves)[22] or by the function that they perform, such as rhythmic segmentation or peristaltic waves. A functional classification of the patterns of waves is certainly to be preferred but at the present stage of knowledge we recommend a classification that does not commit the investigator to an interpretation of the exact mechanical effects of the waves seen in balloon or pressure records and one that may be applied to patterns of motility observed throughout the length of the gastrointestinal tract.

The classification that we have found to fulfill these requirements most satisfactorily is an adaptation of that first proposed by Templeton and Lawson[23] for description of the motor activity of the large bowels of dogs and later applied by Adler, Atkinson and Ivy[6] to motor studies in human beings. The waves seen in balloon and pressure records are simply classified as types I, II or III; recently, it has been necessary to add a fourth category.[24,25] The esophagus is the only part of the alimentary canal in which this terminology does not seem particularly appropriate or helpful.

Type I waves are simple, monophasic waves of low amplitude, usually producing changes in pressure that vary in different portions of the gastrointestinal tract from 5 to 15 cm. of water. Their duration ranges from five to twenty seconds. When present rhythmically, their rate is the most rapid of any of the waves seen and is exceedingly constant and characteristic for the portion of the bowel in which the balloon or pressure unit rests.

Type II waves are also usually simple but are of greater amplitude and duration than those of type I. Their amplitude usually ranges from 10 to 50 cm. of water and their duration from twelve to sixty seconds. When occurring in a rhythmic pattern, their rate in the stomach is equal to that of rhythmic type I waves but in the large bowel their frequency of occurrence, when rhythmic, is slower than that of type I waves and is usually about 2 waves per minute.

Type III waves are complex. Their most important component is a change in base line or basal pressure, often referred to as a "tonus change." Superimposed on this change of basal pressure are waves of types I or II. The duration of the change in base line pressure may range from a few seconds to some minutes and its amplitude from less than 10 cm. of water in the stomach and colon to 30 to 40 cm. of water in the small bowel. In our experience they are seen rather infrequently in the stomach and colon but are a regular component of records of motility in the small bowel.

Studies on patients who have ulcerative colitis have forced the introduction of a fourth category, a type IV wave. This wave occurs only in the colon and, while it has been seen commonly in patients who have ulcerative colitis, it occurs rarely in fasting normal persons.[24,25] The classic type IV wave is a large simple one, simple in that no other components are superimposed on it. Its duration ranges from one to more than two minutes and the pressure that it develops in our balloon system has usually been in the neighborhood of 14 to 15 cm. of water. Type IV waves sometimes occur in rhythmic sequence, and then their rate is 1 every two or three minutes.[24]

QUANTITATIVE ANALYSIS OF MOTILITY RECORDS

In recent years attempts have been made to analyze quantitatively the records obtained from balloon and other recording systems.[6,26,27] A period of observation of at least one hour is usually necessary before a satisfactory estimate can be obtained. Even at slow speeds of recording, voluminous records are thus soon accumulated. It seemed imperative to us that some means of transposing the picture seen in these records to a summarized table was essential for progress in studies in our laboratory. We have tentatively adopted and are currently using the following procedure. The total period of observation is first noted. The proportion of this period in which activity of any type is present in the record is determined and expressed as a per cent of the total time. Then, the per cent

of the time of observation during which waves of types I, II, III or IV are present is separately estimated. The frequency of occurrence and the time during which each type of wave is present in rhythmic pattern are determined separately. Finally, the height and duration of the individual waves are measured.

The objective in developing this method has been to enable an observer to depict the main features of a series of records of motility more accurately from a small table of numbers than if such records were laid lengthwise and reviewed visually. Adoption of the method has enabled comparisons of a quantitative nature to be made in normal persons during control periods and when food or drugs were administered and among normal persons and patients who have disorders of the alimentary canal. Although it admittedly has shortcomings, it has aided in bringing to light factors that would have been missed if purely descriptive methods had been used and it has accomplished quantitative rather than impressionistic summarization of voluminous records.

ESOPHAGEAL MOTILITY

Comparison of Normal Persons and Patients Who Have Cardiospasm. Recent studies of esophageal motility have employed roentgenologic, balloon and pressure-detection technics. Observations have been made on normal persons and on patients who have cardiospasm and scleroderma. In balloon studies by Kramer and Ingelfinger,[8,28] a rubber condom capable of holding 40 cc. was used.[22] This was inflated in the esophagus to a pressure of 20 to 25 cm. of water. Records were obtained with the balloon held in place in the upper, middle and lower parts of the esophagus. The time of transit of the balloon from the upper part of the esophagus to the stomach was determined when it was free to move. The motility studied was initiated by the presence of the inflated balloon and not by swallowing. Next, the movements of the esophagus were observed roentgenoscopically when barium was swallowed,[29] and in the third study, a tiny electric pressure transducer was used to determine changes in pressure in the upper, middle and lower parts of the esophagus associated with swallowing.[30]

When the balloon was inflated in the upper part of the esophagus of normal persons, from fifteen seconds to nearly six minutes were required for its passage into the stomach.[8] The variability of the time required reduces the usefulness of this measurement but the fact that the balloon did reach the stomach indicates the propulsive nature of the motility induced by its presence. When the balloon was inflated and then held in the upper part of the esophagus, the pattern of waves was irregular and characterized by excursions of small amplitude. When the balloon was in the mid-portion, and to a somewhat lesser degree when it was in the lower part of the esophagus, large and well defined waves with a regular rhythm were noted. The average frequency of the waves in the upper, middle and lower parts of the esophagus was 9.5, 6.5 and 8.1 waves per minute, respectively. Their action was definitely peristaltic for they moved the balloon when the tube was free or produced a strong tug on the tube when it was fixed. Roentgenoscopic examination disclosed coincidental rings of contraction passing down over the balloon. Kramer and Ingelfinger suggested, and we concur, that these waves are produced by secondary peristaltic contractions described many years ago by others and recognized in recent roentgenologic examination.[29] These secondary peristaltic waves do not arise as a result of swallowing but as a consequence of esophageal distention. This may be produced, for example, by inflating a balloon, by the passage of large volumes of liquid into the esophagus with or without swallowing or by the production of an obstruction within the esophagus and the accumulation of material and consequent distention of the esophagus above the site of obstruction.

When Kramer and Ingelfinger[8,28] placed their balloon recording system in patients who had cardiospasm, a strikingly different picture was observed. The tone of the esophagus was greatly reduced so that twice or three times the amount of air passed into the balloon under the same pressure that was used in normal persons. In two of the four patients studied the pattern of waves was greatly decreased in amplitude. In the other two, strong phasic activity was present but the pattern was irregular and in none of the patients was the balloon propelled by the action of the waves.

In roentgenologic studies Templeton[29] reaffirmed the recognition of three types of waves in the esophagus of normal persons. He saw (1) primary peristaltic waves initiated by swallowing that started in the pharynx and passed over the entire length of the esophagus; (2) second-

ary peristaltic waves initiated by distention that started at about the level of the aortic arch and were capable of emptying a normally distended esophagus and (3) a localized group of tertiary contractions that were also usually initiated by distention but that occurred principally in the lower third of the esophagus in older persons and were of variable duration and of unknown significance. His description of the secondary peristaltic waves induced by distention with barium fits well the pattern of waves noted by Kramer and Ingelfinger when the esophagus was distended by the balloon.

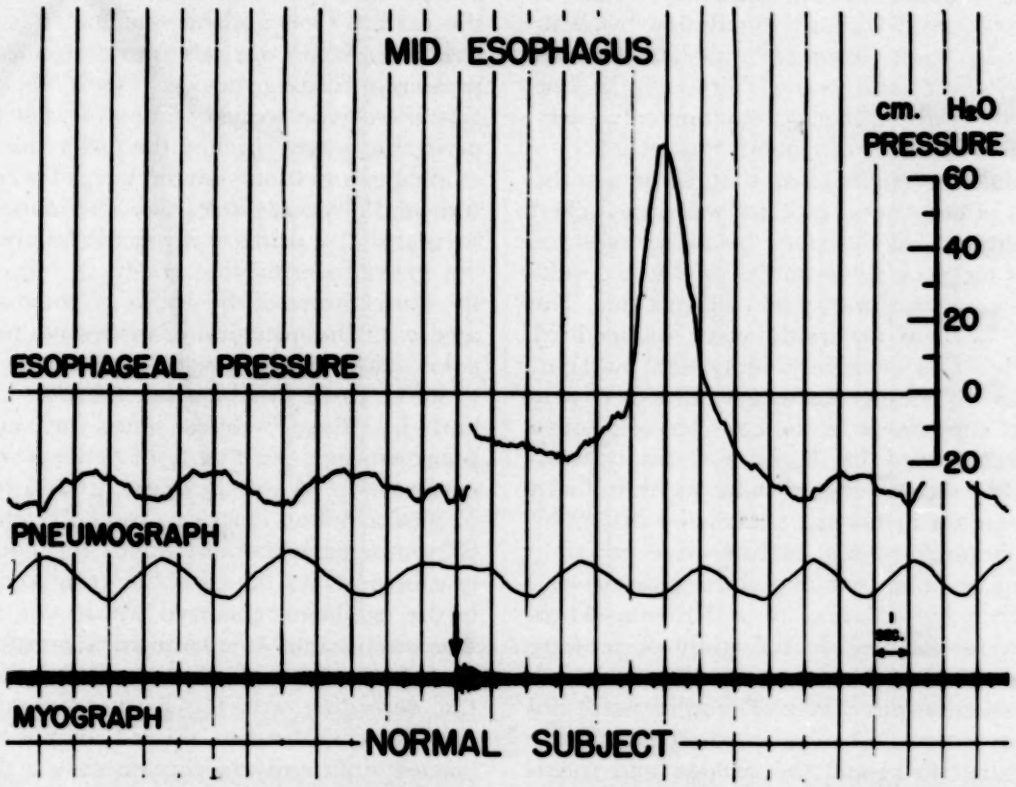

FIG. 2. Pressures recorded by tiny electric transducer in the mid-esophagus when a normal person swallowed at the point on the myographic record indicated by the arrow. Large increase and decrease of pressure occurred as the primary peristaltic wave induced by deglutition swept by the transducer. Vertical lines are one second apart.

A normal primary peristaltic wave induced by swallowing was seldom seen in any of the thirty-nine patients who had cardiospasm and who were studied roentgenoscopically by Templeton.[29] Instead of a wave that proceeded through the entire length of the esophagus as it does normally, Templeton noted that if such a wave were initiated at all in the patients who had cardiospasm it disappeared in the upper portion of the esophagus and in all but three of the thirty-nine patients it was not recognizable below the level of the arch of the aorta. Also, properly co-ordinated, secondary peristaltic waves were not seen. Instead, in all but two patients, purposeless, shallow, segmental contractions constantly appeared and disappeared at different levels of the esophagus. Sometimes, general tonic contractions producing great narrowing of the esophagus accompanied these undulating movements and forced barium into the stomach.

In the study by Butin, Olsen, Moersch and one of us (C.C.),[30] records were made of pressures in the upper, middle and lower parts of the esophagus when persons swallowed saliva, water and cookies. In a group of normal persons a typical and consistent pattern or complex of changes in esophageal pressure was observed in response to deglutition. (Fig. 2.) The chief component of this complex was a large wave of positive pressure that rose rapidly to a peak and declined with similar speed. On the average, this wave reached the upper, middle and lower parts of the esophagus 3.2, 6.3 and 9.7 seconds after swallowing. Its orderly progress suggested

that it represented the advancing, co-ordinated, true primary peristaltic wave. Two decisive differences were noticed between normal persons and patients who had cardiospasm; (1) swallowing usually induced either no complex or an abnormal response and (2) in a considerable number of the patients, waves of motility occurred without stimulation by swallowing. In most instances this spontaneous motility waxed and waned although in one patient the waves occurred rhythmically every twenty-five seconds throughout the record.

It should be emphasized that, although the problem of esophageal motility was approached in the studies just described by means of three different technics, these studies produced results that are complementary and confirmatory. The primary or true peristaltic wave induced by swallowing and decisively delineated by Templeton in his roentgenoscopic studies is clearly the chief component in the complex of positive pressure observed by Butin and his collaborators. The secondary peristaltic waves induced by Templeton in normal persons by distention of the eosphagus with barium were certainly the same as those stimulated by Kramer and Ingelfinger by inflation of a balloon. These waves were not seen in the study of pressure because distention was not produced. In all studies spontaneous waves of motility were observed in patients who had cardiospasm. These waves failed to propel the balloon and roentgenoscopically they appeared to be purposeless. In roentgenoscopic studies and in those of pressure, swallowing by patients who had cardiospasm nearly always failed to induce a true primary peristaltic wave. In all investigations abnormal motility or abnormal responses to swallowing were seen in all parts of the esophagus, indicating that in this disease a defect occurs which extends through the entire eosphagus.

Scleroderma. Kramer and Ingelfinger[8] studied four patients who had scleroderma. Propulsion of the inflated balloon through the esophagus was slow or absent, tone was reduced and the pattern of waves was absent or markedly diminished. Five patients with the same disease examined roentgenoscopically by Lindsay, Templeton and Rothman,[31] displayed defective peristaltic movement when barium was swallowed. When their patients swallowed barium while supine, it remained in the esophagus until they were raised into a sitting or standing position, at which time the bulk of the barium promptly passed into the stomach. No obstruction was apparent at the cardia, and it seemed obvious that the emptying of the esophagus was accomplished by gravity rather than by contractions of its wall. When the patient swallowed while in the vertical position, the barium passed from the pharynx to the stomach without delay at the cardia. One patient who had scleroderma was observed in our laboratory by means of a pressure-recording device. When the patient swallowed repeatedly while supine, few primary peristaltic waves reached the lower third of the esophagus and those that did were of poor amplitude and obviously defective. The investigations suggest that a primary myogenic failure is present in scleroderma that results in relaxation of the musculature of the entire esophagus, associated with the initiation of ineffective peristaltic contractions in response to swallowing.

Studies with Methacholine Chloride. Kramer and Ingelfinger[28] have noted an important pharmacologic peculiarity of patients who have cardiospasm. When they gave from 6 to 10 mg. of methacholine chloride (mecholyl chloride®) intramuscularly to four patients who had cardiospasm, a tonic, lumen-obliterating contraction of the esophagus occurred within two minutes. The contraction counteracted a pressure of 20 cm. of water, forcing air out of the balloon into the recording system. Roentgenographically, even a considerably dilated esophagus contracted sufficiently to contain only a thread of barium. The tonic contraction forced little material through the cardia. Instead, the esophageal contents, whether barium or recording balloon, were displaced into the upper part of the esophagus. At the time of the tonic contraction the patients frequently complained of substernal distress similar to the pain sometimes produced in them by the ingestion of food. In contrast, the normal persons or patients who had scleroderma who were given the same quantities of the drug displayed only a slight increase in esophageal tone. Recent observations in our laboratory are confirmatory.[32] An increase in esophageal pressure has been consistently recorded from the pressure transducer in the esophagus when mecholyl chloride is given to patients who have cardiospasm.

GASTRIC MOTILITY

Studies with Balloons in the Pyloric Antrum of Normal Fasting Persons. The pyloric antrum has been used frequently in recent studies because

small balloons may be conveniently localized there and because this region is usually more active than other parts of the stomach. Records of motility taken from the antrum show three types of waves. (Fig. 3.)

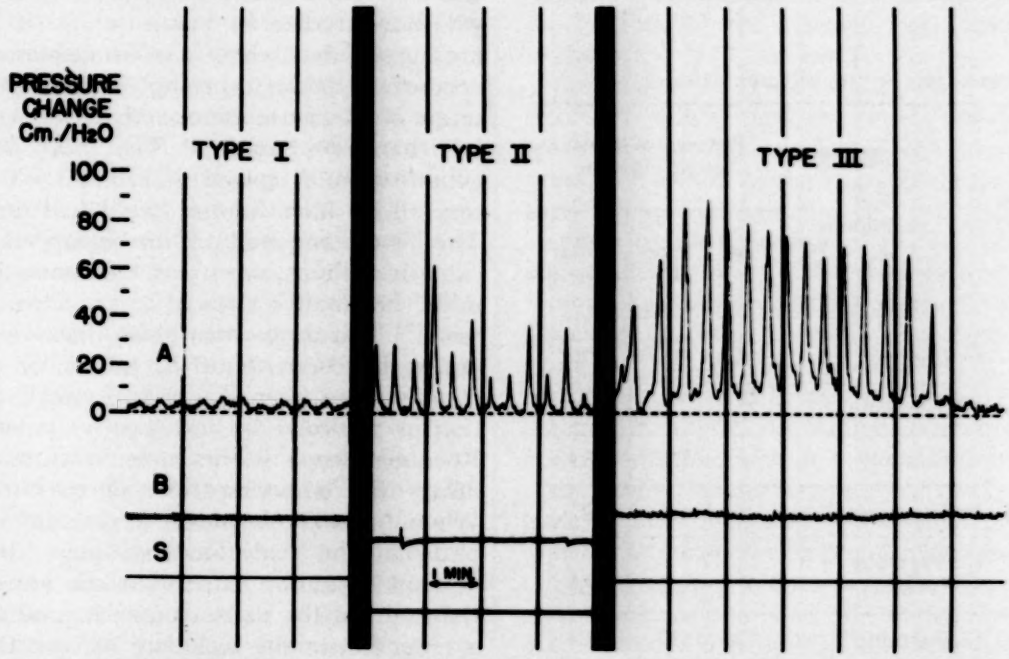

FIG. 3. Types of waves observed in balloon recordings of motility from pyloric antrum of normal human beings. Note that the rhythmic rate of type I and II waves is identical at 3 per minute. The type III wave, composed of a rise in base line pressure on which is superimposed a burst of rhythmic type II waves, is seen infrequently in our records.

The type I waves recorded from the antrum have an amplitude that is equivalent to less than 5 cm. of water and their duration varies from eighteen to twenty-two seconds.[27] They occur in both rhythmic and non-rhythmic patterns and when present rhythmically their rate is constant at 3 per minute. Their function is predominantly mixing in nature.[21] They have been referred to in the literature by such names as "waves of twenty second rhythm," "tonus rhythm" and "mixing waves."

The type II waves have the same duration and rhythmic rate as those of type I but they are always of greater amplitude, developing pressures that range usually from 10 to 50 cm. of water, although heights equivalent to 100 cm. have been encountered. It seems possible that type I and II waves arise as the result of the same basic stimulus, since their rate of occurrence when in rhythmic sequence is identical, and when rhythmic, type II waves may replace those of type I without disturbing the pattern of the sequence.[27] Type II waves are identical with the hunger contractions that were recorded originally by Cannon and Washburn[33] from the main portion of the stomach. They are peristaltic in nature and their function is predominantly propulsive.[18,34]

Type III waves, which are composed of a series of rhythmic type II waves superimposed on an elevation of the base line pressure, have been observed infrequently in our records.

Motor activity of some type was present in the antrum during about 40 per cent of the period of observation in a study of fourteen normal fasting persons. During the remaining 60 per cent of the period of observation, no gastric motility was noted in the records.[27] The active periods were composed of type I and II waves, type I waves being present for about 25 per cent of the observation time and type II activity during 15 per cent. (Table I.)

Duodenal Ulcer. Records of antral motility obtained from small balloons in twelve patients who had unobstructed duodenal ulcer disclosed total activity present for about the same proportion of the observation time as in normal persons, but the distribution of the waves comprising the activity was quite different. Type II (peristaltic) waves were present much more often and type I or mixing waves were seen

much less often.[31] (Table I.) The mean amplitude and duration of both type I and II waves and their rate, when rhythmic, was the same in the patients who had duodenal ulcer as in the normal persons studied previously. When motility from the body of the stomach is recorded,

TABLE I
MOTILITY IN THE PYLORIC ANTRUM

Persons Studied		Test Conditions	Per cent of Time Activity Present		
			Type I	Type II	Total Activity
Normal	14	Control[27]	23	15	38
	11	Neostigmine[27]	22	16	38
Duodenal Ulcer	12	Control[35]	8	26	34
	13	Vagotomy[36]	21	6	27
	12	Vagotomy + urecholine[36]	37	22	59
	12	Banthine[35]	12	12	24
Hypertension	7	Control[37]	11	34	45
	10	Sympathectomy[37]	17	30	47

the patterns obtained from patients who have duodenal ulcer are apparently not distinctively different from those obtained from normal persons.[38]

It has been demonstrated recently that a high degree of correlation exists among the occurrence of a rhythmic, burning, mid-epigastric type of ulcer pain, type II contractions as recorded by a small balloon in the antrum[35] and phasic waves of pressure in the antrum and duodenum as recorded by open-tipped pressure technics.[12] As might be expected, no such correlation has been found with motility as recorded from the body of the stomach.[38]

Drugs That Inhibit Gastric Motor Activity. Cholinergic blocking agents, of which atropine is the classic example, have long been known to reduce gastric motor function. The recent synthesis of substances that have the same type of general physiologic effect but that exhibit a more localized action in the stomach, has reawakened interest in the physiologic and clinical use of such agents.

Most of the compounds studied have belonged to the series of quaternary ammonium compounds. The first of these recently investigated was tetraethylammonium chloride (TEAC). All who have studied its action on gastric motility are agreed that when it is administered intravenously it causes a prompt and complete cessation of all gastric motor activity for something less than one hour.[39–41] The short period of action of the drug and its pronounced circulatory effects have limited its clinical usefulness. The first compound of the group to receive extensive clinical trial was methantheline bromide, hereinafter referred to as banthine® bromide.[42] This drug when given intravenously or orally, has been found to inhibit or produce complete cessation of waves of gastric contraction as recorded by intragastric balloons.[42–44] Roentgenoscopic studies have shown that it also delays the emptying time of the stomach.[45] When banthine bromide was given orally to patients who had duodenal ulcer, the total amount of motor activity in the antrum was reduced but the main change it produced was a reduction in the incidence of type II waves. (Table I.) Coincidental with the disappearance of or reduction in the type II waves, disappearance of intermittent epigastric pain was noted.[35] All studies are in essential agreement. The drug definitely inhibits or abolishes type II contractions. The reduction of these waves, since they are peristaltic, accounts for the slow gastric emptying time noted in roentgenologic studies.

Hexamethylene-1:6-bistrimethylammonium diiodide (hexamethonium iodide®) is the most recent member of this series of compounds to be studied in human beings. When given intramuscularly, it has produced complete inhibition of gastric motility as recorded by balloon kymography in patients who have duodenal ulcer.[46]

Vagotomy. Most of the studies on the effect of resection of the vagus nerves on gastric motility have been made in patients who have duodenal ulcer. All studies that employ balloon methods are in agreement that resection of the vagus nerves is followed by a period of gastric hypomotility.[47–54] The type of wave most frequently absent or present in reduced numbers in balloon records of motility has been the peristaltic type II wave[36,47,49,50,54] and this has been verified by observations with pressure-recording devices.[12] In a detailed study of records from the antrum

it was shown that the total amount of activity in patients who have duodenal ulcer is only moderately reduced by vagotomy but a striking change occurs in the distribution of the waves.[36] Vagotomy increased the incidence of type I waves from 8 to 21 per cent of the observation time. This was equivalent to restoration of the normal amount of this type of activity. The type II waves, on the other hand, were strikingly reduced, decreasing in incidence from an average of 26 per cent of the observation time in patients who had duodenal ulcer without vagotomy to 6 per cent after the operation. (Table 1.)

Roentgenoscopic examinations uniformly have revealed reduced tone, loss of peristaltic contractions and prolonged emptying time in the stomach immediately after operation.[49] Thus complete agreement exists between balloon and roentgenoscopic studies. The reduction of peristaltic type II waves concerned with the emptying of the stomach noted in the records obtained by means of a balloon correlates well with the delayed emptying time and retention of contents of the stomach seen roentgenoscopically. Some studies made three to nine months after operation have shown a return toward normal motor activity.[49,50,55,56] The exact sequence of this recovery has not been established and the results of other investigators have indicated a more prolonged period of disordered motility.[53] The difference may be related to the technic of the operative procedure but further clarification is needed.

Drugs Employed to Counteract the Inhibitory Effects of Vagotomy. Cholinergic drugs have been found most effective in counteracting postvagotomy gastric hypomotility. Of these, urethane of beta methylcholine chloride (urecholine®) and carbaminoylcholine chloride (doryl®) have been the most satisfactory.[52,54,57] Urecholine has been used most often. Under its action, whether administered orally, sublingually or parenterally, the quiescent state of the stomach after vagotomy has been changed to one of decided activity in which peristaltic type II waves have been recognized in both balloon and roentgenoscopic studies.[52,57] In a detailed study of antral gastric motility in vagotomized patients, the amount of activity seen in the records was increased from 27 per cent of the total period of observation in vagotomized patients who did not receive urecholine to 59 per cent when the drug was given.[36] At the same time the type II or peristaltic waves showed an increased incidence of 6 to 22 per cent. (Table 1.) Mecholyl chloride has been found less effective than either doryl or urecholine.[52,53]

Drugs such as neostigmine, which affect the cholinergic system by inhibition of cholinesterase, have been shown to have little or no effect on motility of the normal[27] or vagotomized stomach.[52,53,58] (Table 1.)

Sympathectomy. Three studies have been carried out concerning the effect of bilateral splanchnic section associated with varying degrees of resection of the thoracolumbar sympathetic chains. In one study on two patients an increase in the duration of periods of gastric motor activity and in the number and amplitude of the contractions was observed. This was associated with more rapid emptying of the stomach.[59] In the other two series, which included observations in six cases in one[60] and in ten in the other,[37] no change in gastric motor activity was observed after operation. In one of these studies[37] observations were made on ten patients after sympathectomy, nine of whom underwent the operation because of severe essential hypertension. A control study was made on seven patients who had the same disease but did not undergo sympathectomy. No significant difference was noted between the motility in the control patients and in those who were operated on. It is interesting, however, that the total amount of activity present in the hypertensive patients was somewhat greater than in normal persons. (Table 1.) Both the control patients and those who had sympathectomy displayed a greater amount of type II or peristaltic activity than is seen normally.

MOTILITY OF THE SMALL BOWEL

Normal Pattern. A great many different descriptive terms have been applied to the motor activity of the small bowel as seen directly by roentgenoscopy and as recorded by balloons or pressure-pickup units. The waves of motility recorded from the small bowel by balloon[22,61] and pressure-pickup units[10] may be divided into type I and type III waves. (Fig. 4.)

As elsewhere in the gastrointestinal tract, the type I wave is a small, simple wave. Its amplitude in the small bowel represents a pressure of about 5 to 15 cm. of water, depending on the region from which the record is taken. The duration of the waves varies from 2.5 to 7.5 seconds.[61] When occurring in rhythmic sequence, their mean frequency ranges from 11 to 7 per

minute.[10,61,62] We regard these type I waves as identical to those which have been referred to as "small (S) waves"[22] and as "segmental, ring-like contractions having a nonpropulsive action in the bowel."[26] When rhythmic, they are apparently identical to the rhythmic segmentation seen so clearly in animals.[63]

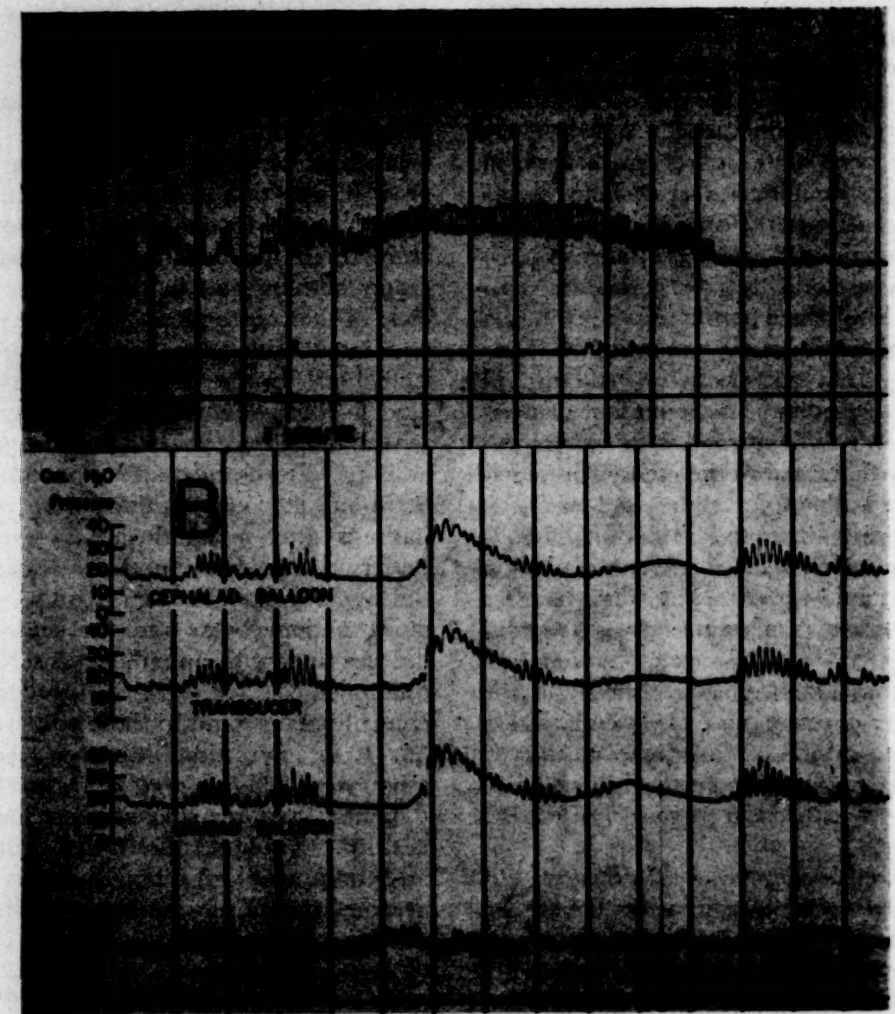

FIG. 4. Motility of the small bowel. *A*, balloon recording. At left is the usual pattern of irregular changes in the base line pressure (type III waves), with irregular type I waves superimposed. To the right is a spontaneous and more pronounced rise in base line pressure (type III wave), with a burst of type I waves in rhythmic sequence superimposed, followed by a quiescent period. This latter complex does not occur frequently. *B*, tandem balloon and electric transducer recordings of ileal motility. Type I and type III waves are present. Note spontaneous and pronounced type III wave in mid-section of record, with burst of rhythmic type I waves superimposed. Rhythmic rate of type I waves in this region of small bowel is slower than that of duodenum.

Type III waves in the small bowel, as elsewhere in the gastrointestinal tract, are complex. In the small bowel they are composed of a change in base line pressure on which is superimposed type I waves. The component that we designate and measure as the type III wave is the change in base line pressure. The mean duration of type III waves ranges from about one half to five minutes and their amplitude ranges from 8 to 35 cm. of water.[61] These waves have been referred to as "large (L) waves,"[22] "tall waves with a broad base," "propulsive peristaltic contractions"[26] or "tonus waves."[61]

In the small bowel the function of the type I wave is predominantly mixing, while that of the type III wave is predominantly peristaltic or propulsive.[22,26] Classic type II waves have not been seen in recordings of activity of the small bowel made in our laboratory.[61]

Under normal circumstances, the small bowel shows much more continuous activity than is evident in the stomach or colon. For example, in a group of normal persons tested after an overnight fast, the upper part of the small bowel was active for 62 per cent of the total observation time.[61] Under similar circumstances, the pyloric antrum is active only about 40 per cent of the time and the colon for even less.[24] (Table II.) In the small bowel, as in the stomach, type I waves normally account for the major portion of the total activity.[61]

Many of the important recent additions to knowledge of motor activity of the small bowel in conditions of health and disease have been made by Ingelfinger and his co-workers.[22,60,64–66]

Sleep. Although many early investigators were of the opinion that sleep had little effect on motility of the alimentary canal, it has been shown recently in man that motor activity of the small bowel is usually depressed by sleep. For example, in less than five minutes after falling asleep, twelve of sixteen persons had a decided reduction in motor activity of the small bowel.[64] The changes in the pattern of motility, however, were not constant. For example, type I waves sometimes decreased in amplitude or frequency and sometimes disappeared entirely. When type III or large propulsive waves were present in the control period they almost always disappeared during sleep. It is clear that sleep should be avoided in studies designed to determine the effects of experimental procedures, drugs or disease on the motor activity of the small bowel and some evidence exists that the same precaution should be taken in similar studies on the large bowel.[6]

Nausea. Ingelfinger and Moss[65] have studied the influence of nausea on the motility of the duodenum in man. Nausea without vomiting was induced by caloric stimulation of the labyrinth or by the administration of morphine sulfate. Motility was recorded from the duodenum by a system of tandem balloons. In eighteen tests in which nausea occurred, the descending duodenum underwent a generalized contraction or "spasm" simultaneously with the onset of nausea and often expelled the balloons into the stomach. Pronounced nausea was characterized by complete obliteration of the duodenal lumen when viewed roentgenographically. The published recordings from the balloons show a pronounced rise in base line pressure, sometimes to the point of complete emptying of the balloon. The records also indicate that when the balloons were not emptied or expelled into the stomach, the characteristic change was that of a rise in base line pressure, on which rhythmic type I waves were superimposed. This type of complex has often been recorded from the small bowel in our laboratory. We have classified the change in base line pressure as a type III wave.

Abbott, Mack and Wolf[67] have made a further study of the relation of this sustained type of contraction of the duodenum to nausea and vomiting. As judged from their illustrations, their balloon system was not emptied by the spasm and their records all show a clear-cut type III elevation in base line pressure, on which rhythmic type I waves are superimposed. Although the duodenal contraction often caused a reflux of the balloons back into the stomach in their tests, as it had in those of Ingelfinger and Moss, both groups of workers were satisfied that it did not represent true reverse peristalsis. Using caloric vestibular stimulation, Abbott, Mack and Wolf noted that, while the complex was often associated with nausea, three persons experienced nausea without this type of contraction and when ipecac was given no correlation was present between nausea and the appearance of the complex. Some persons displayed duodenal spasm during distressful or painful mental and physical situations. The correlation, however, was not particularly high, since five persons experiencing pain had no duodenal spasm, yet spontaneous duodenal spasm was observed on twelve occasions. The authors concluded that although sustained contraction of the duodenum is characteristic of nausea it is not necessarily associated with it and may occur after a variety of distressing but not necessarily nauseating stimuli. A similar, if not identical, type of duodenal complex was observed by Abbott and Pendergrass[68] after the administration of morphine. The same complex has been noted in our laboratory under a variety of normal circumstances during which patients were experiencing neither nauseating nor distressing mental or physical stimulation.[61] (Fig. 4.) Under all of these circumstances, a type III contraction (duodenal spasm) was associated with a burst of rhythmic type I waves. The duration and amplitude of the type III component were usually not so great as when morphine was given or nausea induced. The physiologic basis for the association of a burst of rhythmic type I waves with a type III conductor is unknown.

Obstruction. Balloon kymographic studies by Ingelfinger and Abbott[22] have shown that as a recording balloon approaches an obstruction in the small bowel it registers increasing tone, a gradual disappearance of type I waves and an increase in the frequency and amplitude of type III waves; immediately proximal to the site of the obstruction, in the decompensated segment of the bowel, all activity ceases.

Direct determinations of intraluminal pressure have been made during surgical operations in patients who had intestinal obstruction, by the insertion into the bowel of a large needle connected to a water manometer. In five cases in which obstruction of the small bowel was present, sustained pressures ranging from 4 to 18 cm. of water were recorded. During peristaltic waves this pressure increased up to 30 cm. of water.[69]

The studies suggest that frequent, powerful, type III peristaltic waves occur in the small bowel in response to distention. Tests employing this reaction to distention might be useful in the elucidation of the mechanisms of a disordered bowel.

Sprue. Ingelfinger and Moss[66] have carried out repeated kymographic balloon studies of motor activity of the small bowel in two patients, one of whom had tropical and the other non-tropical sprue. In both cases the bowel offered much less resistance to increases of volume in the balloon than was noted normally. Before treatment was instituted, large type III or propulsive waves were not seen in either case. Type I, non-propulsive (S) waves were present and occurred with a normal frequency of 12 per minute, but their amplitude was diminished. The rate at which the balloon was moved along the small intestine was only slightly slower than usual and its transit occurred even during periods when type III (L) waves were absent. In both cases, a tendency was present for the recording to return toward a normal appearance after weeks or months of therapy.

Two patients who had non-tropical sprue were studied recently in our laboratory. In both patients reduced amplitude of the non-propulsive type I waves was noted, with preservation of their usual rate of occurrence when in rhythmic sequence. Type III waves were also present although they, too, were of less amplitude than usual.

The absence or reduction of propulsive type III activity offers an explanation for the delay in transit of barium through the small intestines in patients who have sprue.[70–72] The diminution in both type I and III activity may be related to the altered absorption of liquid from the bowel of such patients when digestion is in progress.[73] Up to the present, however, no evidence exists of a direct correlation between any of the forms of waves seen in studies of motility and absorption from the gastrointestinal tract. Such studies should be profitable and are needed.

Acute Dysentery. Roentgenoscopic determinations of the time of transit of barium through the small bowel have been made in fourteen patients who had acute bacillary dysentery. A definite delay was noted in nine patients, in spite of the fact that many continued to have frequent bowel movements during the examination.[74] The finding of hypopropulsion in the small bowel in a diarrheal state should prompt a search for the precise type of motor function that is disturbed in this condition.

Action of Drugs. Placebos: It has been shown that propulsive type III waves and the total activity in the small bowel may decrease as much as 32 and 24 per cent, respectively, after the administration of placebos.[75]

Inhibitors: Both atropine and tincture of belladonna given orally have been shown to inhibit the motility of the small bowel. They produce a reduction in the total activity and in the incidence of propulsive type III waves.[76]

All of the recently synthesized quaternary amines that have been tested on the bowel have been found to be effective inhibitors. The first of these, tetraethylammonium chloride (TEAC), usually eliminates temporarily all types of motor activity in the small bowel.[77–79] Banthine bromide also inhibits duodenal motor activity.[44] Roentgenoscopic tests have shown that its administration likewise delays the time of transit of barium through the small intestine.[45,73]

Stimulators: Balloon and roentgenoscopic studies have demonstrated that neostigmine[26] and urecholine[72,80] are effective stimulators of motor activity of the small bowel. In one study, neostigmine was found to increase the incidence and amplitude of type III propulsive contractions.[26]

Morphine: All are agreed that morphine diminishes the time of transit of material through the small bowel but its action is complex, for it generally causes an increase of tone[68,81] that may be spasmodic,[82] together with an increase in the amplitude of non-propulsive waves. How-

ever, it always produces a pronounced decrease in propulsive type III contractions.[68,81,82] The suggestion has been made that it has an un-coordinating effect on the motor function of the bowel so that propulsion is less likely to occur.[7,81]

irregular or rhythmic patterns. (Fig. 5.) They appear in records made by both balloon and pressure-pickup systems and, with the balloons used in our laboratory, their dimensions are identical in the two types of recordings.[10,20] In

TABLE II

TYPES OF GASTROINTESTINAL MOTILITY IN FASTING NORMAL HUMAN BEINGS (MEAN VALUES)

Portion of Gastrointestinal Tract	Type I				Type II				Type III*			Total Activity, Per cent
	Per cent Time Present	Rate per Minute When Rhythmic	Amplitude, cm. of Water	Duration, Seconds	Per cent Time Present	Rate per Minute When Rhythmic	Amplitude, cm. of Water	Duration, Seconds	Per cent Time Present	Amplitude, cm. of Water	Duration, Seconds	
Stomach:												
Cardia	..	2	6	30	..	..	..	..	..	..	...	..
Body	..	3	2	20	..	3	23	15	..	..	...	..
Antrum	23	3	<5	20	15	3	30	15	<1	7	120	38
Small Bowel:												
Upper part	62	11	17	5	..	..	..	..	36	8	25	62
Lower part	..	7	5	8	..	..	..	..	..	35	120	80
Pelvic Colon	1	13	5	5	36	2	9	25	2	3	90	36

* Rhythmic rate was not recorded.

Type IV waves have occurred so infrequently in normal individuals that their characteristics cannot be reported accurately at present.

Ineffective Agents: Most of the so-called antispasmodics, such as amprotropine phosphate (syntropan®), 2-diethylaminoethyl 9-fluorenecarboxylate hydrochloride (pavatrine hydrochloride®), diethylamino ethyl phenyl thienyl acetate (asymatrine®) and 2-diethylaminoethyl diphenylacetate hydrochloride (trasentine hydrochloride®), when given in the recommended dosages, have not produced greater alterations in the motility of the small bowel than those that are often seen after administration of placebos.[83,84]

MOTILITY OF LARGE BOWEL

Normal Pattern. (Table II.) The most complex patterns of waves encountered in balloon recordings of motor activity are obtained from the colon. The classification presented here has been tentatively adopted in our laboratory. Modification is clearly envisaged as correlations with roentgenographic observations and physiologic functions are established. Classification on a functional basis with a more appropriate nomenclature should be possible later.

Type I Waves: General agreement exists that type I waves are simple and small and occur in the pelvic colon, their mean duration is five seconds and their mean pressure is about 5 cm. of water.[10,24] When such waves are recorded higher in the colon, they usually last longer and are of greater amplitude. Their duration, however, seldom exceeds twelve seconds and their amplitude is usually not more than 12 cm. of water. When rhythmic, their rate in the pelvic colon is about 13 per minute,[24] whereas in the descending colon it may range from 3 to 8 per minute[6] with a mean of 6 per minute.[10] Throughout the colon type I waves often occur as one component of a type III complex. However, they are not seen frequently in recordings from the lowest parts of the large bowel and in this respect they differ from their cousins in the small bowel. For example, in the rectosigmoid of a group of fasting normal individuals these waves occurred during only about 1 per cent of the total time of observation,[24] while under similar circumstances in the small bowel they were present for about 60 per cent of the time.[61] All are agreed that these waves are non-propulsive.[6,24,25] As far as we can ascertain, these waves have never been recognized by direct observa-

tion or by roentgenoscopic examination of the human colon.

Type II Waves: The type II wave that is most readily recognizable in recordings of colonic motility is also simple but of much greater dimensions than the type I. (Fig. 5.) It is usually shown in its simple form in published illustrations. Examples are Figure 2*d* and *e* of Adler and associates,[6] Figure 2 of Spriggs and coworkers[24] and Figure 1 (top tracing) of Kern and associates.[25] However, in the classic paper of Adler, Atkinson and Ivy,[6] the type II wave was described and represented schematically as a rapid rise and fall of base line pressure with superimposed type I contractions giving the appearance of what we term the "Christmas tree wave." This type of wave is seen particularly in records showing pronounced activity. Under such circumstances this mixed type II wave, if it does represent a true type II wave, may be exceedingly difficult to differentiate from a short type III wave. The difficulty has not been resolved and it remains one of the problems to be dealt with when making detailed analyses of records.

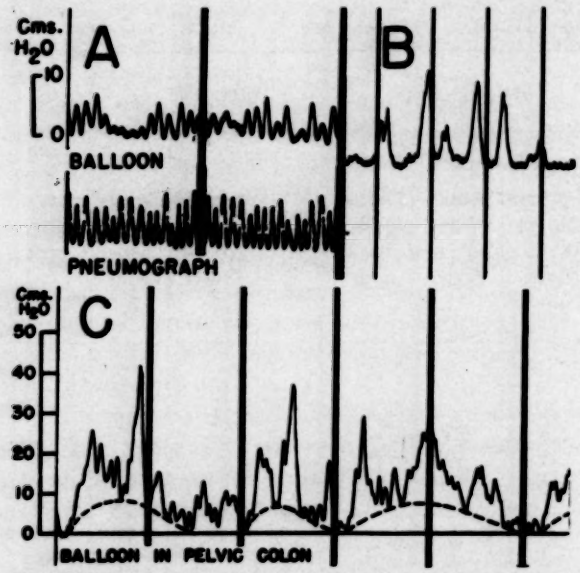

FIG. 5. Balloon recordings from pelvic colon of normal persons. Vertical lines are one minute apart. *A*, type I waves; *B*, type II waves; *C*, type III waves. (Composite of illustrations from Spriggs, E. A., Code, C. F., Bargen, J. A., Curtiss, R. K. and Hightower, N. C., Jr. Reproduced with kind permission of the editor of *Gastroenterology*.)

Type II waves from the lower portions of the colon have been studied in detail in our laboratory. Their average duration has ranged from about twenty-five to thirty seconds.[10,24] Their mean amplitudes in the rectosigmoid in two studies have been 6.5 and 9.7 cm. of water, though waves developing pressures up to 50 cm. of water have been encountered. In the descending colon, the pressures recorded by type II contractions are on the average about twice those recorded in the pelvic colon.[10] The exact dimensions of the waves from other regions of the colon have not been reported.

Type II waves comprise the major activity seen in records from the pelvic colon.[24] In this region they often occur as bursts during which they sometimes assume a rhythmic pattern of about 2 waves per minute.[24]

The pelvic colon of normal fasting persons shows some form of activity about 36 per cent of the time and nearly all of this is composed of type II activity.[24] Apart from the occurrence of mass movements, some indication is present that the total amount of activity in the colon may be increased by feeding[6,85] but detailed analyses of records of colonic motility obtained during fasting and then after eating are not available.

Our present view is that type II contractions are the cause of the constrictions in the large bowel that produce the haustra seen by the radiologist. They probably also caused the haustral contractions traced by Schwarz[86] on serial roentgenograms of the large bowel. The contractions he traced occurred in all parts of the large intestine but were most powerful in the proximal portions. We have noted that the amplitude of the type II contraction is greater in the descending than the pelvic colon. These contractions noted by Schwarz were also studied by Barclay[87] by means of roentgenocinematography and his work again points to the similarity between hustral contractions and type II waves.

Recently, contractions of a similar nature have been observed directly by Grace, Wolf and Wolff[88] in exposed segments of the large bowel of unanesthetized human beings. The contractions were described as localized, circular or annular constrictions usually involving less than 25 mm. of the exposed segment of colon. The circular constriction often divided the exposed bowel into separate segments but it was not described as progressing over the exposed segment and the authors were of the opinion that groups of such contractions would be manifest in roentgenograms as haustrations. Neither radiologists nor those who have viewed these contractions directly have defined their precise temporal characteristics; general de-

scriptions, however, fit closely the pattern of type II waves seen in balloon and pressure recordings.

Radiologists have demonstrated that the haustral contractions are not propulsive peristaltic waves.[86,87] Barclay,[87] using pills of bismuth, has clearly shown that haustral contractions move colonic contents from side to side and backward and forward over short distances in the colon but are not concerned particularly with moving the contents analward; rather, their function is that of mixing and turning the contents over. The action of type II waves, as recorded by balloon and pressure systems, suggests that they have precisely this function.[24,25] These classic type II or haustral contractions, therefore, seem to be performing a somewhat similar function to segmentation as observed in the small bowel.

Adler, Atkinson and Ivy,[6] in their original application of the classification of type I, II and III colonic waves to human beings, stated that some type II waves resulted in propulsion of fecal material and some did not. They stated that type II contractions of low amplitude were non-propulsive in nature and were not perceived by the persons being studied. In this and a later paper,[7] they developed the concept of co-ordination between the motility of adjacent segments of bowel. They suggested that if, for example, a type II contraction occurred in a proximal segment and was followed or integrated with a similar contraction in the distal segment, propulsion of material would occur. They observed this type of activity about 5 per cent of the time in persons who had recently been fed. If a type II contraction in the proximal segment was not integrated or co-ordinated but was disorganized in relation to the distal segment, no propulsion would take place. This was found to be the most common type of activity present in their records of normal persons. Their paper, however, does not include illustrations of records in which this type of progressive and co-ordinated segmental activity is shown.

In a study in our laboratory in which a system of tandem balloons was used, this concept of co-ordinated type II contractions was adopted and the type II waves that were observed were divided into either co-ordinated or un-co-ordinated contractions, depending on the time of occurrence of the waves in the consecutive segments of the bowel in which the balloons rested.[89] Although this concept may be quite correct, for the time being at least its use has been discontinued in our laboratory because it was found on re-examination of the records that the waves which had been tabulated as co-ordinated type II contractions occurred simultaneously in both balloons of the tandem system as in Figure 2 of McMahon, Sauer, Bargen and one of us (C. F. C.).[89] These waves, therefore, could not represent a haustral contraction that had been initiated in the proximal segment and had then passed to the distal segment but must have been due to simultaneous activity in both segments. Later, this was brought out clearly in a study of normal persons and patients who had ulcerative colitis.[24] It then became apparent that the contractions tabulated as co-ordinated type II waves most likely represented a different type of activity. Therefore, such waves have been separated into a fourth category. It is possible that some or most of the propulsive, co-ordinated, type II waves in the records of Adler, Atkinson and Ivy also belong in this fourth type. Even if this were true, we do not consider that the concept of co-ordinated activity of type II waves should be abandoned.

Our studies have been directed mainly to activity in the lower part of the colon. Consecutive type II contractions occurring first in a proximal segment and then in the adjacent distal segment may constitute an important type of activity in other portions of the colon and under circumstances we have not studied. Statistically, such action is bound to occur a certain number of times just by chance and the possibility of its purposeful occurrence should always be borne in mind during analyses of balloon or pressure records.

Type III Waves: The description we employ of this wave is precisely that given by Adler, Atkinson and Ivy.[6] It is a complex pattern of waves composed of a change in base line pressure or a change in tonus on which are superimposed type I or type II waves or both. (Fig. 5.) In our experience, their duration usually ranges from about one to four minutes. However, they have not been frequent or conspicuous in records obtained from the lower part of the colon in our laboratory.[10,24] In one group of fasting normal persons, they were present about 2 per cent of the time of observation in records obtained from the pelvic colon.[24]

In detailed analyses of records of colonic motility, the height and duration of the change in base line pressure have been measured and recorded as the type III wave. The super-

imposed components have been considered separately in their appropriate categories. In normal persons, the pressure developed by type III waves has usually amounted only to about 3 to 5 cm. of water in the pelvic colon and to 8 to 10 cm. of water in the descending colon. The duration of type III waves is usually a matter of minutes.[10,24]

The type III wave has not been seen apparently by radiologists. At least no adequate description of it has been found in the radiologic literature. The small amount and slow rate of change of pressure associated with this wave would render it almost imperceptible by roentgenoscopic or even serial roentgenographic examination. Its function is not established though all are agreed that it is not peristaltic.[6,25] The prolonged duration of the type III wave suggests that it may have something to do with absorption, possibly aiding it by increasing the hydrostatic pressure within the gut.[90] Little other evidence supports such a hypothesis except the finding that this wave does not seem to be associated with propulsion and it obviously is not a mixing wave.

Type IV Waves: Studies by means of balloon and pressure recording technics in patients who have ulcerative colitis have forced the inclusion of a fourth category of wave recorded from the colon.[24,25] In records from the descending[91] and the pelvic colon[24] of patients who had this disease, large, smooth waves apparently produced by a single component were often seen. The only other waves of simple composition that we were accustomed to encounter in records of colonic motor activity were types I and II, yet the waves seen commonly in records obtained from patients who had ulcerative colitis produced on the average more than twice as much pressure as type I or type II waves, and their duration, instead of being a matter of seconds, was customarily two or more minutes. Also, when in rhythmic sequence, they did not have the frequency of 13 per minute of type I waves or of 2 per minute of the type II waves, but they occurred at a rate of about 1 wave every two or more minutes. Finally, in patients who had ulcerative colitis, these waves were often associated with a feeling of fullness in the rectum, a desire to defecate or the passage of fecal material. They were definitely propulsive in nature. They were, therefore, designated separately as type IV waves.

It seems most probable that waves previously classified in our laboratory as co-ordinated type II waves[89,91] were indeed type IV waves. When stimulated by neostigmine, these waves appeared in a rhythmic pattern, "neostigmine rhythm," at a rate of 1 per two minutes.[89] The waves from each balloon of a tandem system occurred simultaneously in the records and they must have represented the rhythmic contraction of a portion of the bowel at least 12 to 14 cm. long. (Fig. 6.) Roentgenoscopic studies of this rhythmic activity are needed.

The disappearance of other forms of waves in the presence of the type IV wave, its definite propulsive action and its dimensions, clearly associate it with the mass movement of the colon noted many years ago by radiologists. This type of activity in the large bowel was first decisively described by Holzknecht in 1909.[92] He saw the movement in only 2 of more than 1,000 roentgenologic examinations of the colon. He recognized that during the contraction the haustra disappeared, and the colon assumed a smooth, sausage-like outline over a considerable distance; this was followed by the rapid transport of the contents of the affected portion into a more distal section of the colon. He noted that the actual transport of material was accomplished in a few seconds. He suggested that in normal persons only three or four such movements occurred in a day. His observations were verified by Barclay[93] and extended by Hurst and Newton,[94] who showed that the mass movement was most often associated with the taking of food. The original concept of the contraction was that it represented a peristaltic wave sweeping through a segment of the bowel.[92,94,95] Studies with tandem balloons indicate that this is not the case. Contraction of the affected portion of the bowel occurs, for all practical purposes, simultaneously throughout its length. (Fig. 6.) A number of segments are involved. In most recordings a rapid upsweep of pressure is noted, after which a slower decline of pressure takes place. The latter limb of the wave represents its main temporal component. (Fig. 6.) It seems likely that the movement of colonic contents occurs during the rapid upsweep of pressure. This would account for the sudden transport of material seen roentgenoscopically. Once the bowel is emptied, its relaxation might not be evident roentgenoscopically and if slow, as in the pelvic colon, it would account for the gradual decline in pressure in a balloon lying in the affected segment.

During direct observation of exposed segments of human colon, Grace, Wolf and Wolff[88] have seen motor activity that likely represents type IV contractions. They have observed a generalized constriction and shortening up to 50 per cent in the length of exposed segments of large bowel, causing the affected portion to assume a tubular shape without superimposed annular contractions. This type of activity was seen to occur during defecation.

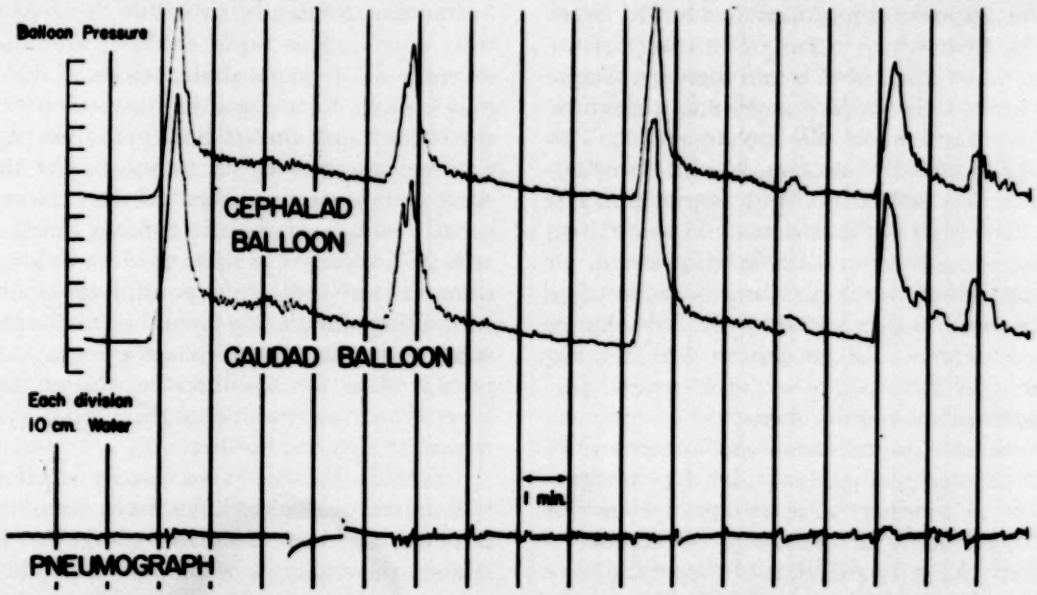

FIG. 6. Type IV waves occurring every five minutes in the transverse colon and detected by a system of tandem balloons. These waves were formerly classified as coordinated type II waves. Note, however, simultaneous occurrence in both balloons, indicating contractions are not propagated peristaltic waves but mass movements involving a number of segments simultaneously.

It appears evident, therefore, that type IV waves represent classic mass movements in the human colon. They occur so infrequently in normal persons during the course of a day that they are generally missed, even during prolonged periods of observation. They deserve further study, particularly regarding their possible relation to the co-ordinated type II activity described by Adler, Atkinson and Ivy.[6] The development of methods that will stimulate their occurrence in the normal colon would aid such studies.

Emotion. Almy and his associates[96–99] carried out a series of investigations concerning the effect of emotion on motor and other functions of the large bowel in normal persons and in patients who had spastic constipation and functional diarrhea. Motor activity in the pelvic colon was observed directly through the proctoscope or studied by means of balloons placed through the proctoscope in the pelvic colon. Pain was induced by placing the subject's hand in ice water (cold pain) or by tightening multiple screws held by a special device around and across the top of the subject's head (head pain). Emotional reactions, without physical stimulation, were induced by discussion of life situations known to evoke emotional conflicts in the individual.

Proctoscopic examination disclosed that cold pain and head pain increased the contractions of the colon in seven healthy medical students.[96] A more extensive study of healthy persons by means of balloon kymographic recording methods, demonstrated that considerable increase in the contractile state of the pelvic colon occurred in only about half of the tests in which pain was induced by cold or by compression of the head or in which troublesome life situations were discussed.[97] In each of these positive tests, the increased motility of the colon was associated with a generalized physical and emotional reaction which indicated that the individual was under stress. In the remainder of the tests, activity in the pelvic colon did not change and in only two of these instances did signs of a general reaction to stress develop. Similar results were obtained in patients who had spastic constipation.[98]

Rather different responses were obtained in patients who had irritable colon (functional diarrhea), eight of whom had diarrhea alone, seven constipation alone and three alternating

constipation and diarrhea.[99] During the control period, the tracings showed a steady pattern of complex waves. This is unusual in our experience, since activity is not often continuous in the lower part of the colon. During the discussion of emotionally charged topics, two distinct and opposite alterations in motility were seen. The first, which occurred in many of the patients, was represented by an increase of tone and an increase in the frequency and amplitude of the contractions of the pelvic colon. This pattern was usually associated with an emotional reaction of hostility and aggression. The second, which all patients showed at some time, was represented by a sudden diminution or disappearance of wave-like contractions, often associated with a decrease in tone. This change appeared suddenly in association with the development of an attitude of hopelessness, personal inadequacy or self-reproach.

It is difficult to tell from the records published in this series of papers which type or types of waves of motility were stimulated by the stressful situations. In one series of records[98] a burst of rhythmic type II waves appears to have been stimulated by an emotional dilemma in the patient.

Some of the results of Almy and his associates have been confirmed by Grace, Wolf and Wolff.[88,100] These workers found in two patients who had exposed portions of the colon that the occurrence or discussion of life situations that produced emotional conflicts involving pronounced feelings of anger, anxiety, apprehension, resentment, hostility or guilt, were associated with increased motor activity of the exposed colon and secondly, that situations provocative of abject fear, fright or dejection were associated with hypofunction of the colon, illustrated by relaxation and a lack of contractile activity.

No quantitative analysis of the records has been presented in any of these studies. The incidence of increased activity occurring spontaneously or of reduced activity occurring without a change in mood or emotion on the part of the patient is not stated. When records that have been obtained from normal persons lying quietly in a soothing physical and mental atmosphere are examined consecutively so that many hours of recordings are represented, one is struck by the phasic occurrence of colonic motor activity. Periods of activity often alternate with periods of inactivity and the duration of both the active and the inactive periods is extremely variable. This point was early emphasized by Adler, Atkinson and Ivy.[6] They pointed out that the amount and quality of colonic motility in normal persons is as variable as it is in the dog and that not only do the different segments vary in regard to the type of motility they manifest at any particular time but the type of motility is subject to rapid change. They noted in recently fed persons that periods of quiescence may endure for as long as sixty minutes or, on the other hand, that periods of activity may last for one hundred eighty minutes. The theses of Almy and Grace and their co-workers would be greatly enhanced if their data included the results of prolonged periods of observation on the same persons when no painful or emotionally conflicting stimuli of a mental or physical nature were introduced, and if some estimates were presented of the likelihood of the changes observed during stress arising from a purely coincidental change in motility.

Ulcerative Colitis. Two recent studies using balloon recording technics have been made in patients who had ulcerative colitis.[24,25] In both studies the balloons were placed in the pelvic colon and in both instances two unusual features were seen in the records. In many of the patients type IV waves occurred frequently. They were almost uniformly associated with propulsive action, leading to expulsion of the balloons or of fecal material. Also, in a considerable number of the records, practically no motor activity was visible except for a few scattered type IV waves. In one of the studies[24] a detailed analysis was made of records from ten fasting patients. Compared to tracings from a similar series of normal persons, the total amount of activity present in the records of the patients who had ulcerative colitis was just half that observed in the normal series. Type I activity increased from 1 to about 3 per cent, but the significant changes in the records were a decisive reduction in type II activity, a complete lack of type III waves and the presence of type IV waves. In the larger group of cases studied[25] not all the patients who had ulcerative colitis displayed such pronounced changes in motor activity. Nevertheless, the generalization seems justified that the majority of patients who have had this disease for a period of years display a reduction in the total amount of activity in their colons, accompanied by a shift from mixing and absorption-promoting types of motility toward a propulsive, excretory type of activity.

Congenital Megacolon. Swenson and his coworkers[101–103] have shown recently that when small amounts of barium are allowed to trickle into the rectum under continuous roentgenoscopic examination, a narrowed segment of the rectum and rectosigmoid, up to 10 cm. or more in length, becomes evident in patients who have congenital megacolon, particularly when they are viewed from the oblique position. They regarded the narrowing as indicative of spasm,[101] although turbulent and purposeless motor activity with occasional reversed peristalsis was sometimes noted in the narrowed segment. In three patients motility in the narrowed segment was recorded by means of a balloon.[103] In one of these patients no contractions occurred, in another, rhythmic segmentation was noted and the third displayed normal contractions that were independent of activity in the upper dilated segment of bowel. Recording the contractions of this narrowed segment of bowel by means of three balloons at different levels, Hiatt[104] found in several patients that the motility was caused by mass contractions of the entire segment. The roentgenoscopic, balloon and postcolostomy observations of Swenson and his collaborators suggested to them, as pathologic studies had suggested to others,[105-106] that the narrow segment presented a region of functional obstruction in the colon. They found that this narrowed region corresponded to segments, previously recognized by others,[105,107] in which the ganglionic cells of the myenteric plexus are reduced in number or entirely absent. The concept of functional obstruction in this narrowed segment, presented by Swenson and associates, was verified by the results of resection of the involved portion. Their ingenious operation has been found to restore normal bowel function in a great number of persons in whom it has been performed.[101–103,108]

The similarity of the movements of this narrowed segment of the lower end of the colon in patients who have congenital megacolon to those of the lower end of the esophagus described in patients who have cardiospasm[29] suggests a basic parallelism between the two conditions.[105] The finding that mecholyl induces a pronounced spasm in the lower end of the esophagus in patients who have cardiospasm suggests that the effects of this drug on the lower portion of the colon should be tested in patients who have megacolon. Other studies on the reactivity of the narrowed segment by means of balloon and pressure-detection technics should be productive, since it is possible that the smooth muscle in this portion of the bowel is present in a "pure" state or at least is free from the influence of local ganglionic cells.

Studies with Drugs. As already mentioned, the large bowel normally shows periods of motor activity and inactivity of varying length. It is, therefore, exceedingly important, when assessing the action of a drug, to obtain sufficient control observations to establish, under the conditions of the tests, the amount of activity present when the drug is not given. A second factor, stressed by Grace, Holman, Wolf and Wolff[109] is that the responses of the colon to drugs may depend on whether the action of such drugs reinforces or opposes that of other forces, such as the emotions, already acting on the colon.

Inhibitors: The classic and the more recently developed cholinergic blocking agents, such as atropine, tetraethylammonium chloride and banthine bromide, all predominantly inhibit motor function in the large bowel.[7,78,83,109,110]

Stimulators: The parasympathomimetic agents, neostigmine and mecholyl, stimulate motility of the large bowel.[89,109,111–113] In a detailed study of the types of activity affected by neostigmine McMahon, Sauer, Bargen and one of us (C. F. C.[89]) found that its chief effect was to alter the character of the motility of the large bowel from a predominantly non-propulsive to a predominantly propulsive action. The number of type I and type III contractions decreased during the action of the drug, while the number of type IV waves was increased. The results, in general, confirmed earlier observations.[113]

Morphine: Atkinson, Adler and Ivy[7] have shown that morphine, like neostigmine, has its predominant effect on the distribution of the types of activity in the colon. Its effect on the total amount of activity present may, like that of neostigmine, be slight. Its over-all effect, however, is exactly opposite to that of neostigmine. These workers have demonstrated that morphine increases the amount of non-propulsive motility and definitely decreases the proportion of propulsive activity present in the colon. They found that it also increases the tone of the large bowel.

Ineffective Agents: A large number of so-called antispasmodic agents, such as methylamino-isooctane (octin), pavatrine, syntropan, trasentine and phenobarbital, when administered by mouth in the recommended therapeutic doses,

have been found to have no effect on motility of the large bowel.[88,109,110]

CONCLUDING COMMENT: HOPE FOR THE FUTURE

A classification of the types of motor activity in the alimentary canal that is based on the function which each section performs would aid tremendously in clarifying and advancing studies of motility. Further investigations of the correlation between patterns of motility as recorded by balloon and pressure-pickup units and motor action as observed roentgenologically must be made before assignment of specific motor effects can be made to all types of waves. Also, much work will be necessary before the physiologic action of each type of wave is thoroughly appreciated. At present, only some simple and rudimentary associations can be made.

It is likely that waves of motility perform two general functions, namely, the mixing and the propulsion of the contents. It is also likely that they participate in a third function, namely, absorption. In the performance of these functions, the actions of the different types of waves no doubt overlap considerably. For example, mixing waves, while aiding digestion, will at the same time promote absorption by altering the surface of the contents presented to the mucosa. Likewise, propulsive motor activity, while accomplishing the excretion of unused materials, will aid absorption by spreading the contents over the surface of the mucosa. Both types may promote absorption by increasing intraluminal pressure. Also, declining gradients of mixing activity or of pressure-producing activity down the digestive tract would cause progress of liquid contents.

However, even the proper assignment of its predominant function to each type of wave would greatly aid progress in this field. Changes in motor activity of the alimentary canal associated with disease could then be precisely described and delineated in terms of the motor activity most significantly affected. If the effects of drugs on each type of wave of motility were also known, the physician could administer agents whose actions would be specifically directed toward correction of the precise derangement of motor action present in the patient.

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Combined Staff Clinic

Antigen-antibody Reactions

These are stenotyped reports of Combined Staff Clinics of the College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York. The clinics, designed to integrate basic mechanisms of disease with problems of diagnosis and treatment, are conducted under the auspices of the Department of Medicine. The reports are edited by Dr. Gilbert H. Mudge.

Dr. William B. Sherman: I am sure you are all more or less familiar with the fact that antibodies, which are a mechanism of immunity, can contribute under certain circumstances to the symptoms of the patient. For one simple example, if a mouse is injected with rabbit antipneumococcus serum, it is well protected against infection by pneumococci but will react with anaphylactic shock to an injection of the specific pneumococcus polysaccharide which is harmless to a non-immunized mouse. The field of application of antigen-antibody reactions to clinical disease is very broad and we can cover only a very small segment of that field.

Several of the various ways in which antigen-antibody reactions can be involved in disease have been discussed quite recently in other meetings such as the experimental encephalomyelitis apparently related to sensitization to homologous tissues, and the possibility or probability that antigen-antibody reactions may play a part in rheumatic fever and glomerular nephritis. In view of the limited time allowed we will omit these fields entirely and devote our attention primarily to reactions in which a specific substance introduced into a patient gives rise to a reaction which either is a demonstrable antigen-antibody reaction or in which there is good reason for believing it is related to such a mechanism. Dr. Kabat will begin by reviewing some of the fundamentals of anaphylaxis in its typical form.

Dr. Elvin A. Kabat: When antigen and antibody combine *in vivo* one can, in various species and under varying conditions, obtain a number of so-called allergic reactions. In not all of these various allergic reactions has the mechanism by which the reaction takes place been completely elucidated. However, there are a few such reactions which are better understood. Of these I will discuss two very briefly, namely, anaphylaxis and the Arthus reaction.

Anaphylactic shock occurs when antigen is introduced into an animal which has previously either produced antibody itself in response to an earlier injection of antigen (active anaphylaxis) or which has artificially received antibody by injection of antibody-containing serum (passive anaphylaxis). Unfortunately, the amount of antibody produced by an animal which has been given a sensitizing dose of antigen bears no relation whatever to the amount of antigen which it received. Animals show an enormous range of variation in response to a uniform dose of antigen so that one has no way of knowing how much antibody has been produced by the sensitizing injection. This makes it difficult or impossible to determine how much antibody and antigen are necessary for anaphylaxis to occur. But since we have available methods developed by Dr. Heidelberger and his coworkers over the past twenty-five years for measuring antibodies in serum, it is a simple matter to inject known amounts of antibody into a normal animal, then inject antigen and determine how much antigen is required to induce anaphylaxis. The guinea pig is the animal of choice.

If groups of guinea pigs weighing about 250 gm. are injected intravenously with increasing known amounts of antibody nitrogen, for example, anti-egg albumin in amounts ranging from about 0.002 mg. N to about 0.07 mg. N, and forty-eight hours later an intravenous injection of antigen is given, the severity of the anaphylactic response increases with the quantity of antibody used for sensitization. With the smallest amounts of antibody no appreciable reaction occurs. As one increases the amount of antibody given to each guinea pig the incidence of reactions increases and they become more severe. With larger doses anaphylactic death ensues and by the time one reaches about 0.03 mg. antibody N per 250 gm. guinea pig one can sensitize all the animals and produce uniformly fatal anaphylactic shock; similar

results have been obtained using rabbit antibody to Type III pneumococcus polysaccharide. It is evident that only extremely small amounts of antibody need be present in the tissues of an intact animal to produce allergic reactions. Consequently, the detection of very small amounts of antibody is one of the important problems in allergy. In many types of allergic reactions we have no way of knowing the amounts of antibody required because we do not have *in vitro* methods for measuring them.

One might interpose the objection that, after all, *rabbit* antibody was used to sensitize a *guinea pig* and that perhaps species differences may play a role—guinea pig antibody might behave quite differently in the guinea pig. In experiments designed to test this possibility, however, the same quantity of guinea pig anti-egg albumin (0.03 mg. N) was found to be required for uniformly fatal anaphylaxis. With a high molecular weight antigen such as anti-tobacco mosaic virus the same quantity of antibody N is needed for sensitization but larger doses of antigen are required to induce fatal shock.

If one takes from a female guinea pig which has received a uniformly fatal sensitizing dose of antibody, i.e., 0.03 mg. antibody N, a strip of uterine muscle and places it in a Dale bath, it will contract when antigen is added. The uterine strip may weigh approximately 75 mg. in relation to the 250 gm. of guinea pig. If one assumes (and it is a pretty fair assumption from the available data) that the 0.03 mg. antibody N is fairly uniformly distributed through the tissue, by taking the ratio of 75 mg. to 250,000 mg., and multiplying by 0.03 mg. N, one computes that this strip of uterine muscle has been sensitized to give a uniformly maximal contraction after having received about 1/100,000 of a mg. of antibody N. This amount is much smaller than could possibly be detected by any of the usual methods.

Many of the problems with respect to human allergic reactions may involve antibodies in such minute quantities. As if the problem were not difficult enough on this account, it turns out that antibodies in whole serum are of several different types. There are certain antibodies which will precipitate when antigen is added. There are other antibodies which are present in antisera and do not by themselves precipitate with antigen. They may be detected, however, by a rather simple experiment. Suppose one takes an antiserum to crystalline egg albumin containing 1.0 mg. antibody N per ml. If instead of adding the quantity of egg albumin required to precipitate all of the antibody in one portion one adds a fraction of this amount of egg albumin in small portions, let us say 7, 8 or 10 successive small portions to the serum in a tube, the first addition will produce a precipitate. One can centrifuge that off, make a second addition, a third, a fourth, etc. Eventually a point where precipitation no longer occurs will be reached. At that point, if all of the antibodies in the original serum were the same, one should have removed the entire 10 mg. antibody N. Usually such is not the case; at the point at which one of these small successive portions fails to precipitate one may have removed only about 8 mg. antibody N. So there are unaccounted for, shall we say, 2 mg. antibody N. These 2 mg. can be detected in the supernatant serum after it no longer precipitates with antigen alone by adding to a portion of it some fresh whole serum, and then adding antigen. This type of antibody may be thought of as non-precipitable antibody because after the precipitating antibodies in the serum are removed it cannot by itself precipitate, but in the presence of precipitating antibodies it can attach itself to a precipitate and be carried down and measured.

If in terms of the framework theory of antigen-antibody reactions one considers the antibody which precipitates in serum to be bi- or multivalent, the reaction of antigen and antibody produces a huge aggregate of multivalent antigen which react with multivalent antibody and it is this huge fabric which precipitates from solution. The non-precipitating antibody would be an antibody molecule with but one reactive group per molecule, or a "univalent" antibody in terms of the framework theory of immune reactions. This does not mean that this is the only explanation. It is best if one wishes to avoid any firm adherence to the framework theory to describe the findings empirically and term the residual antibody non-precipitable antibody. One can measure the non-precipitable antibody by adding fresh antibody and antigen. One can dilute this antibody and inject it into a guinea pig and see if anaphylactic shock is produced. In careful comparisons of the non-precipitable antibody with the original antibody in whole serum it has been found to be just as effective in producing anaphylaxis as the mixture of antibodies in whole serum.

This raises another problem. Were many of the antibodies in human allergy of this non-precipitable variety, even if they were present in sufficiently large amount, it would not be possible to detect them by the usual *in vitro* tests.

Another reaction which has been measured quantitatively is the Arthus reaction. The Arthus reaction may be considered essentially a reaction in which the combination of antigen and antibody forms a precipitate, damages vascular endothelium and causes various pathologic manifestations which I am not competent to discuss. But if one injects varying known amounts of antibody and antigen, one can demonstrate a relationship between the amount of antibody and the severity of the Arthus reaction. Studies with Dr. Fischel have shown that intracutaneous injection of about 0.025 mg. rabbit anti-egg albumin N into rabbits followed by antigen thirty minutes later gives a minimal Arthus reaction. With larger quantities of antibody the severity of the local reaction increases. The Arthus reaction, however, is much less sensitive to variation in antigen concentration. Larger quantities of antibody are required if administered intravenously. Studies with Benacerraf have established the relationships between antibody and antigen needed to induce an Arthus reaction in the guinea pig. By proper choice of dosage and of the time interval between sensitization and shock, complications due to anaphylaxis may be avoided. The Arthus reaction, unlike the anaphylactic reaction, can be shown to require the formation of an actual precipitate causing the blood vessel damage; because if one prepares non-precipitable antibody, which is as effective as antibody in whole serum in producing anaphylactic shock, it does not produce an Arthus reaction.

Another piece of evidence in this connection is that horse antipneumococcus antibody which, for a reason we do not understand, is incapable of producing passive anaphylactic sensitization in the guinea pig, will precipitate with the pneumococcus polysaccharide and will cause an Arthus reaction in the guinea pig. So we have both sides of the picture, a non-precipitating antibody producing anaphylaxis but failing to cause an Arthus reaction, and a non-anaphylactively sensitizing but precipitating antibody producing an Arthus reaction.

The problem is a very difficult one. It is one of reasoning from analogy. It is essentially trying to get a pattern for the allergic reactions in which we can measure amounts of antibody and trying to determine the relative quantities of antibody and types of antibody which are essential for producing certain of these allergic reactions so that one can better understand the reactions of human allergy. Dr. Sherman has recently reported that with rabbit anti-egg albumin sera which would passively sensitize human skin, giving a Prausnitz-Küstner type of response, the antibody was of the non-precipitating variety. It is through extension of studies of this type that we may increase our knowledge of the different types of allergic reactions and make it possible to compare them, one with another, in terms of the quantities and kinds of antibody necessary for their production.

DR. SHERMAN: The same general type of reaction that Dr. Kabat has described in experimental animals is familiar in clinical medicine as a result of injecting protein antigens, particularly heterologous antisera, into patients. We have very little occasion to inject chemically pure antigen into patients. More often the material injected is a foreign serum which is a mixture of many antigens. Therefore we are unable to follow through with exact quantitative technics, but it is possible to show a very close analogy. Dr. Beatrice Seegal will discuss some of these reactions which take place with the therapeutic use of heterologous serum.

DR. BEATRICE SEEGAL: It is the purpose of this discussion to touch on three aspects of serum disease; the clinical manifestations, the relation of antigen-antibody reactions to the disease picture and the occurrence of concomitant vascular damage.

Serum disease follows the injection of a foreign serum, usually therapeutic horse serum. There are two types of reaction which may ensue. The first, known as *serum shock*, develops almost instantaneously and is characterized by substernal oppression, apprehension, dyspnea, cyanosis, coughing, nausea, vomiting and diarrhea. Collapse may follow and in very rare instances death occurs.

The second type of reaction which may follow injection of a foreign serum is known as *serum sickness*. In this disease there is an incubation period. Generally about one week elapses before the symptoms are manifest, although they may come in a few hours or they may be delayed for two or more weeks. The disease is characterized by skin eruptions, wheals, erythema and purpura; by hot, swollen and painful joints; by an

enlarged spleen and lymph nodes; and, in a minority of cases, by proteinuria and eosinophilia. The migratory polyarthritis has been compared with that found in rheumatic fever. Swift and Boots have shown that the fluid recovered from the joint cavity in patients with serum sickness closely resembles that from the joints of patients with rheumatic fever.

Both manifestations of serum sickness are associated with a demonstrable antigen-antibody reaction. Serum shock in man occurs in a previously sensitized individual and hence is comparable to anaphylactic shock in animals. The sensitization may have arisen from a previous injection of serum, or it may have developed spontaneously as in the natural horse asthmatics. These individuals are sensitive to horse dander; and since there are common antigens between horse dander and horse serum, they also are sensitive to horse serum. Thus the symptoms of serum shock are initiated by the reaction between preformed antibody and the injected antigen.

The relation of serum sickness to an antigen-antibody reaction is not so readily apparent. Previous sensitization usually cannot be demonstrated. Mackenzie and Hanger pointed out that the incidence of the disease is related to the amount of serum injected. When amounts of horse serum up to 10 cc. are administered, only about 10 per cent of patients develop serum sickness. If as much as 100 cc. of horse serum is injected, only about 10 per cent of patients escape the disease. Von Pirquet and Schick in 1905 demonstrated that serum sickness occurs at a time when antibodies to the foreign serum develop in the patient's circulation, and they suggested that the disease is precipitated by an antigen-antibody reaction. Mackenzie and Leake followed the rate of disappearance of injected serum and the development of specific antibody in the circulation of a number of patients, of whom some acquired serum sickness while others escaped. These investigators cited a typical instance to illustrate the clinical manifestations and immunologic course of the disease. A boy aged thirteen, who received 180 cc. of antipneumococcus horse serum, developed the first signs of the disorder four days after injection and during the next fifteen days urticaria, edema, fever, painful joints and enlarged spleen and lymph glands followed. Examination of the patient's serum for the presence of injected horse serum showed that at the onset of the disease this antigen was present in the patient's circulation and that it slowly decreased in amount until at the time of recovery it had disappeared. At the same time search for the presence of antibodies to horse serum demonstrated that in the latter part of the disease these antibodies were reaching high titers. It would appear that the termination of serum sickness in this patient was associated with final elimination of the antigen by these antibodies. In cases in which serum sickness failed to develop, Mackenzie and Leake found that the horse serum was very slowly eliminated. At the end of their period of observation, which was as long as two months, there were large amounts of horse serum still circulating and no demonstrable antibody to horse serum. The absence of serum sickness was thus correlated with the persistence of antigen, the lack of antibody and hence the lack of an antigen-antibody reaction.

A foreign serum such as horse serum, which contains many antigenic proteins, gives rise to as many specific antibodies. The apparent coexistence of horse serum and of antibodies to horse serum in the circulation of the patients studied by Mackenzie and Leake is due to the different rates at which antibodies develop to the various fractions of the horse serum. Overlapping of antigens with non-specific antibodies occurs.

A study of serum sickness in an individual injected with a single antigen would be very instructive since it would permit accurate analysis of the relation of the disappearance of antigen and the development of antibody to serum sickness. Such data are available. During the war, studies were carried out at the Columbia Research Service at the Goldwater Memorial Hospital on crystalline bovine albumin as a possible plasma substitute. Volunteers were injected with this serum fraction. A small percentage of the patients developed serum sickness during the course of the experiment. Dr. Forrest E. Kendall, who studied the disappearance of the antigen and the development of antibody in these patients, has furnished the data recorded in Figure 1 which illustrates the fate of antigen in two patients, one of whom had serum sickness. The amount of albumin in the circulation in grams per cent was determined by the quantitative precipitin reaction over a period of weeks following injection of 25 gm. of crystalline bovine albumin in each of these patients. Patient C failed to develop any serum sickness.

As in most of the other subjects studied, the injected bovine albumin left the circulation slowly. Its half-life was twenty-four days and traces were still observable in the circulation at the end of 140 days. Patient R developed serum sickness on the twenty-second day following the injection of the bovine albumin. The disease lasted for seven days. It may be seen that during the period of the disease the concentration of bovine albumin in the circulation dropped rapidly from 14 mg. per cent to zero. It was then possible to demonstrate .026 mg./ml. of antibody N in this patient's serum. There was thus a correlation between the development of serum sickness and the loss of antigen from the circulation. The development of antibody was demonstrable as convalescence set in.

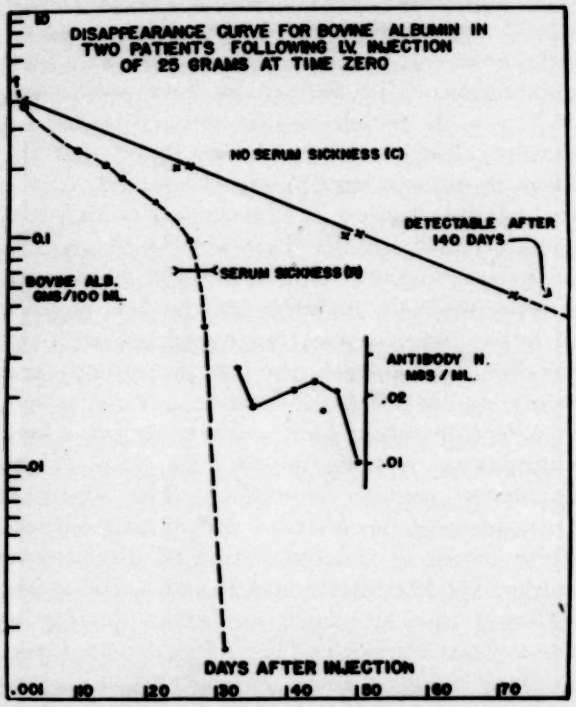

FIG. 1. Antigen-antibody titers in serum sickness due to bovine albumin. (Courtesy Dr. Forrest E. Kendall.)

Serum sickness was formerly considered an annoying rather than a serious malady. However, Rich found in autopsies on individuals who died following a recent attack of serum sickness typical fresh lesions of periarteritis nodosa, characterized by hyaline degeneration and necrosis of the intima and media associated with infiltration of leukocytes. Rich produced such lesions experimentally by injecting rabbits with large amounts of horse serum, 10 cc. per kg. of body weight. Upon sacrificing the animals three to four weeks after this procedure he found severe arteritis and periarteritis similar to that seen in the human cases. Some of these animals had lesions in the heart valves and in the myocardium which Rich considered similar to those found in acute rheumatic fever.

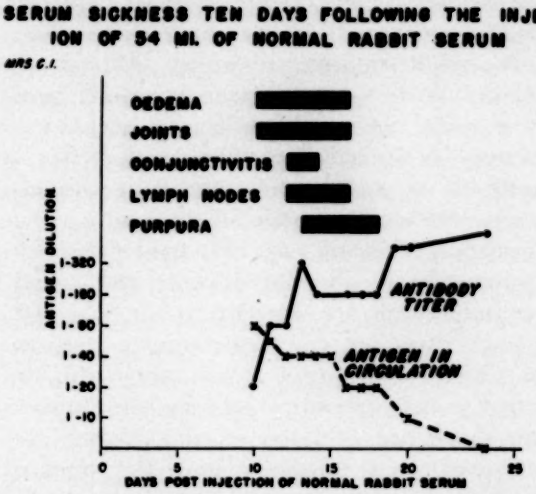

FIG. 2. Experimental serum sickness in the dog.

Since it is customary to show patients in a medical clinic, a case of serum sickness will be presented. Here is Mrs. Cornell's Ivy, a pure bred beagle, who received a total of 54 ml. of normal rabbit serum on March 22nd, 23rd and 24th, during her fourth week of pregnancy. On April 3rd, ten days later, she was found to have typical full-blown serum sickness. Her joints were so swollen and painful that she walked with great difficulty. She had other classical symptoms such as edema of the face, neck and extremities, enlarged axillary nodes, conjunctivitis, and purpuric eruptions, particularly noticeable on the ventral surface of the body about the nipples. Figure 2 indicates the duration of the various symptoms of serum sickness which Mrs. Cornell's Ivy manifested. It may be seen that her clinical course was similar to those which Mackenzie and Leake reported for their human subjects. Her immunologic status is also recorded. On the tenth day she had both rabbit serum and antibodies to rabbit serum in her circulation. During the course of her disease the antigen decreased in the circulation and the antibodies to the rabbit serum rose; and when she was recovering from her disease, her blood contained almost no antigen but still had a high titer of the antibody.

DR. SHERMAN: For a good many years we have been accustomed to thinking of antigens

in terms of proteins. However, for a period of fifteen or twenty years there have been extensive studies by Dr. Landsteiner, Dr. Chase and others at the Rockefeller Institute showing that certain rather typical types of antigen-antibody reactions may be produced by substances of non-protein and relatively simple chemical character.

As an outgrowth of these studies we have also acquired knowledge of a different type of antibody or a different type of transfer of antibody from the actively sensitized to the normal individual. Dr. Chase of the Rockefeller Institute has very kindly consented to come and tell us about some of these studies.

DR. MERRILL CHASE: Without benefit of detailed evidence on slides I want simply to review some of the phenomena that have been seen while travelling another road that leads into the same territory—the area of antigen-antibody reactions. This route was opened through the development of experimental drug sensitization, first carried out successfully abroad in 1928 and 1930 and then developed by Dr. Landsteiner, whose group came to include myself. The extension in chemical thought contributed by Dr. Landsteiner was the happy selection of several sensitizing chemicals for which analogues could be obtained and with which specificity studies would be possible. I came to adopt the course of first studying the guinea pig, as the animal of choice for drug sensitizations, in order to find what occurred with the onset of hypersensitivity in this species, then secondarily, and only then, of learning whether the conclusions may be applicable to human beings. There are, indeed, some differences between the skin reactions of the two species.

The primary task, undertaken by Dr. John Jacobs, was to develop methods of effecting dermal sensitivity. In human studies one may locate sensitized individuals in the populace who will permit one to do specificity tests. In contrast, those who use animals must learn to develop the desired sensitizations in normal populations. (We may say at the outset that most of the sensitizations of which I shall speak are commonly designated as the "delayed type" because the inflammatory reaction, after application of the allergenic material to the sensitized individual, follows a particular course: it becomes visible only after the lapse of four to seven hours, gets progressively more intense with its maximum generally at twenty-four or forty-eight hours, and then gradually fades away.)

Perhaps we can get quickly at the methods employed. First, I wish to emphasize the importance of heredity in determining the degree to which an animal can be rendered sensitive. By selective breeding of either susceptible or resistant animals, it is possible to obtain offspring whose sensitivity will vary in accordance with general Mendelian principles. Thus animals coming from a stock that is sensitizable to a given excitant, will, if subjected to a suitable course of treatments, all become sensitive, although there will be minor individual qualitative differences.

We may remark in passing that it has not yet been possible to desensitize animals once they have been sensitized. But—a fact of theoretic interest at least—it has been found that if the animals are fed with the specific incitant prior to the sensitizing procedure, a new situation arises: the animals fail largely or wholly to respond to later sensitizing procedures carried out with the same chemical. Feeding, it may be remarked, is not the sole method of administration by which profound differences in response may be ensured; further, the time elapsing between the feeding and the attempt at sensitization may be rather long (at least half a year), without loss of the acquired resistance. It can, consequently, be important as to how the individual *first* encounters allergenic chemicals.

One other observation should be mentioned. Drs. Landsteiner and Jacobs, using the guinea pig, were able to bring evidence for the combination of certain sensitizing drugs with the tissues of the body as an explanation of the drugs' allergizing capability. This was accomplished by using compounds consisting of a benzene ring with simple chloro- and nitro-substituents, of which there are many possible isomers. The various compounds available to them were examined for their sensitizing capacity in animals: those able to sensitize were thus detected and set apart from whose which could not sensitize. When the chemical properties were investigated, it was found that the possession of a labile chlorine atom or a labile nitro group was a characteristic of all those compounds that possessed the sensitizing property. The compounds could therefore unite with organic bases; when such union occurred *in vivo*, as with proteins, the new complexes could be

viewed as antigens that were partly derived from the host and partly from the radical of the sensitizing chemical. We may find it useful to designate complexes of this sort as "derivative" antigens.

With this much as introduction we may proceed to a review of the information that has been acquired by studying the allergic guinea pig. In the first place, we have found in guinea pigs brought into a state of sensitivity that there is present in variable measure, along with their delayed type of hypersensitivity manifested upon contact with the chemical in question, also circulating antibodies. These antibodies can be demonstrated by a technic analogous to the Prausnitz-Küstner reaction, with use of young, normal guinea pigs as the recipient animals, and also by passive transfer of anaphylaxis in the way that Dr. Kabat has just described. It turned out, however, and not unexpectedly in view of the concept of "derivative antigens" mentioned previously, that in developing both the Prausnitz-Küstner reactions and the anaphylactic syndrome it was often necessary to use not the simple drug but a preformed "derivative antigen" or conjugate, in which the drug was combined with a suitable protein as a vehicle, e.g., the proteins of homologous serum, or casein or other soluble protein. In this way the use of such conjugates allowed us to find circulating antibodies in cases in which otherwise we could not find them at all. If one examines the literature for instances of drug sensitization in the human being that have been associated with circulating antibodies (usually demonstrated by the passive transfer procedure of Prausnitz and Küstner), we find that the positive reports nearly always have to do with chemicals that are readily reactive with tissue proteins, e.g., phthalic anhydride, chloramine-T and the like; and it does not seem far fetched to assume that, if and when more suitable conjugates are employed, the presence of circulating antibody may be detected in many more types of drug sensitization in man.

In the second place, there is surprising evidence that the white cells play a prominent role in mediating the hypersensitive state, that is, in preparing for reactions of the "delayed type." For example, if white cells are collected from sensitive animals and are washed and injected into normal animals, a state of hypersensitivity ensues which seemingly is not to be duplicated if one gives serum or fractions of serum that are especially rich in antibody. It has so far been found that the cells themselves must be maintained living. The test material that elicits the reaction on the recipient animals is precisely that which has been used to sensitize the group of donor animals. The onset of sensitization in the normal animal is within two to two and one-half days when one injects the cells into the peritoneal cavity; it may come as early as sixteen to nineteen hours if the cells are injected into the blood stream.

Let us consider the following experiment. A normal animal has received white cells from animals made sensitive to ortho-chlorobenzoyl chloride. A few hours later a site on the skin was freed of hair by clipping and a drop of olive oil containing ortho-chlorobenzoyl chloride was allowed to fall onto the skin. For comparison, the same application was made on another animal that had not received the white cells. A positive contact reaction developed by the following day on the cell recipient only. A new area was then freed of hair and a second contact reaction was developed on this new site. On the other flank of the animals an application of olive oil containing in solution another, chemically unrelated sensitizing chemical demonstrates, by the absence of reaction, that the positive contact reaction has the attribute of *specificity*.

Likewise, in the case of tuberculin sensitivity positive transfer effects are to be elicited by cells; the reaction exhibited by the recipient animal when tuberculin is injected intracutaneously is not intense but it has the essential features of the tuberculin reaction. An experiment was done with pooled cells taken from a number of guinea pigs that had been sensitized with heat-killed tubercle bacilli suspended in hydrocarbons. The pooled cells were washed and divided into two portions. The one recipient was given half of the final cell suspension intraperitoneally, the other was given the remainder but not until it had been heated at 48°C. At appropriate times tuberculin was injected intracutaneously. On the day following the first testing there were positive, albeit incipient, reactions on the guinea pig that received the living cells, none on the other. When the testing was repeated and one more day had elapsed, the animal that had received heated cells was still entirely negative whereas both test sites on the animal that had been given the living cells were positive and indubitably so. It may not

be amiss to remark that the proper time interval, and not the repetition of the testing, is the reason for the observed change.

The white cells, then, play a special role that is not yet elucidated. The white cells can be got from the circulating blood, as the leukocytic cream or "buffy coat"; they may be obtained from spleen or taken from the lymph nodes. They may be secured in exudates caused to appear in the peritoneal cavity by the injection of paraffin oil. Lately we have been using principally cells of splenic origin and cells teased from lymph nodes.

Many attempts made to detect circulating antibody in such recipients of white cells were fruitless. Finally, however, after some further years of investigation and improvements in technic, antibody was detected. The antibody was indeed oriented specifically toward the substance that had been employed in the sensitization of the cell donors, it was capable of sensitizing guinea pig skin to undergo an evanescent, local reaction, and it was capable of rendering guinea pigs passively anaphylactic.

Immediately there will come to mind the work of Erich and Harris, and of White and Dougherty relating the lymphoid cells to the production of circulating antibody. Such would be, were it capable of substantiation, a very pretty explanation of the mode of onset of dermal sensitivity after the transfer of white cells: these would carry or give rise to antibody, and the presence of circulating antibody would explain allergic states. There are certain difficulties, however, in the acceptance of this view, apart from the ostensible difficulty that the antibodies in donors' sera do not transfer sensitivities of the delayed type: I refer to quite tentative experiments in which it appears that the two effects following transfer of cells are dissociable.

The cells do not appear to convey significant amounts of preformed soluble antibody, for when white cells of the sensitive guinea pig were ground with sand we could recover no antibody in the extracting fluid. Upon closer analysis we found that the conditions necessary for the finding of antibody were these: (1) the cells had to be living; (2) it required time, usually two or three days, before antibody was detectable in the circulation following cell transfer and, once found, the concentration was seen to increase up to the fifth or sixth day. One could therefore think that the antibody was a consequence of active sensitization. But apparently this is not the case: the antigen that we are using is so poorly antigenic that if it is injected directly into a normal animal antibodies will not be found for fourteen to eighteen days. In contrast, we have obtained antibodies as early as twenty-seven hours after the injection of cells.

We may illustrate these statements. Recordings were made of the behavior of both uterine horns of a female guinea pig that had received, a few days before, cells removed from the lymph nodes of sensitized donors. When the conjugated antigen, in this instance picrylated casein, was placed in the bath, both horns simultaneously swept up in contraction. After the strips had relaxed and the chamber had been washed, a renewal of the same dose of antigen failed to cause a response (specific desensitization) although the tissues were still viable as shown by contraction upon addition of histamine.

The appearance and accumulation of antibody in the blood stream can be followed by means of a series of ear bleedings on the individual recipients of donated white cells. The relative concentration of antibody can then be determined on small samples of serum by means of simultaneous comparison in a recipient guinea pig, with use of a modified Prausnitz-Küstner type of test: equal volumes of the various bleedings (or dilutions thereof) are injected into the skin at different sites and, one day later, antigen is injected intravenously. The reactions appear and are read within three to eight minutes. If the animal chosen as recipient of the cells is a female, individual uterine horns can be removed at operation whenever desired and a comparison made between the results of both sorts of test.

As a further extension of our work, we have found that if cells from highly sensitive guinea pigs are deposited delicately in normal skin, the sites of deposition being regarded as areas prepared for Prausnitz-Küstner testing, local reactions at the sites can sometimes be secured when antigen is injected intravenously. Apparently one must attempt the testing only after an interval of time, say two or three days after deposition of the cells. Whether this procedure simply reveals traces of antibody in the cell mass that are not discernible when the cells are subjected to thorough grinding, or whether it indicates some ongoing process analogous to that by which antibody is found in the circulation of

FIG. 3. Constricted bronchus in allergic reaction to typhus vaccine. (Courtesy of Army Institute of Pathology.)

animals that receive larger volumes of cells, is a question not yet resolved.

A final word is in order regarding the relation of the findings made in the guinea pig with some preliminary observations in human subjects. In the same way as was shown first in the laboratory animal, white cells taken from the tuberculin-sensitive human being are reported by Dr. Lawrence of New York University Medical College to render a tuberculin-negative person tuberculin-positive. In addition, and in the field of human reagins, Drs. Walzer and Glazer have recently reported that cells taken from people exhibiting flare-and-wheal reactions to allergens and possessing corresponding circulating reagins will, albeit at a considerable cost in response in the quantitative sense, cause normal recipients to exhibit local flare-and-wheel reactions at the cutaneous sites of deposition. It remains to be determined whether this is simply residual reaginic antibody already existing in the cells and mechanically transferred with them.

In summary, it may be well to repeat that the transfer of washed white cells from sensitive guinea pigs to normal individuals of the same species results in one or both of the following effects. If the donors have had a dermal sensitivity of the "delayed type," the recipient will come to exhibit the same sort of sensitivity. If the donors have been actively producing or have been prepared so that they are about to produce circulating antibody, the presence of this antibody will be found in the recipient animal. It would appear that the cells involved are "stem cells" that participate in the immunologic process, the particular role that they play if transferred to another animal depending upon the way in which the antigenic or allergenic stimulus has been applied to the donor individual in effecting its sensitization. The situation is evidently complicated, yet it is capable of analysis.

DR. SHERMAN: The work that Dr. Chase has described has helped a great deal in the understanding of allergic reactions to drugs and medications that we see clinically. However, as will soon be apparent, the development of knowledge of the immune mechanisms and types of antibodies involved in these clinical reactions is far behind the experimental work.

We do see clinically very definite and typical examples of both the immediate anaphylactic and the delayed type of reaction that Dr. Chase has described. Also we see a great many drug reactions which in our present state of knowledge are hard to fit into the picture.

Coincident with the decline in the use of heterologous antisera, due to the introduction of the antibiotics, a number of new protein-containing medications have been introduced and we occasionally see typical anaphylactic shock due to the injection of such protein-containing drugs as liver extract, virus or rickettsial vaccines made from egg yolk, and insulin. Reactions to the virus and rickettsial vaccines usually occur in patients who are naturally sensitive or allergic to eggs, and in this way they resemble the serum reactions of horse asthmatics that Dr. Seegal has mentioned.

Figure 3 illustrates the type of reaction which may be observed in a patient who is sensitive to egg yolk following injection of a vaccine derived from egg yolk. This is a section from a soldier in the last war who concealed from the Army authorities the fact that he had always been allergic to eggs. He got by all right until he was given a dose of typhus vaccine which was prepared from egg yolk and had a very prompt fatal reaction. The slide is a section of a bronchiole showing very marked constriction of the lumen, which is further obstructed by secretion of mucus. The constriction of the lumen was presumably due in part to spasm of the circular musculature and in part to the swelling of the mucosa but in any case sufficient essentially to close off the lumen and produce asphyxial death.

With the more commonly used substances, such as insulin, fortunately the reactions are

rarely this severe. Allergy to insulin is relatively common but is usually manifested by urticarial eruptions. A certain number of reactions are specific to beef or pork and by changing the type of insulin it is possible to avoid the reaction. However, there is another group we see relatively frequently in which all types of insulin, including the purest available crystalline insulin, produce the reaction. These are of particular interest in that a certain small number of the patients showing allergic reactions to insulin as an antigen are also resistant to the hormonal effect of insulin. The two conditions may occur separately but occasionally occur together. One patient who was treated in the metabolism ward two or three years ago had urticaria due to insulin and required doses up to 1,500 units a day, which still did not entirely control the glycosuria. The serum of these patients contains a factor distinct from the sensitizing antibody, which can be shown to protect mice against insulin shock. We have no conclusive proof that this is an antibody but its specificity suggests that it probably is an antibody. In the patient who was treated here it was possible to show that the serum would protect mice against all types of commercial insulin but would not protect mice against human insulin. This species specificity is the strongest evidence that it might be an antibody.

Similar anaphylactic reactions, occasionally severe and sometimes fatal, also are noted with a number of non-protein drugs: arsphenamine, quinine, rarely penicillin and, rather interestingly, thiamine, which is a normal and essential constituent of the body. However, when large amounts of thiamine, 100 mg. or more, are injected either intramuscularly or intravenously, it is not unusual for patients to develop a sensitization which may result in an anaphylactic reaction to subsequent injections. In these cases the skin tests with thiamine usually show an immediate urticarial reaction. Occasionally one sees reactions following the oral administration of sulfonamides that are so sudden and severe that they may be considered anaphylactic in type. This, however, is not one of the common reactions to sulfonamides. In an occasional case of this type it is possible to demonstrate a skin-sensitizing antibody to the sulfonamide by the Prausnitz-Küster reaction.

In one instance serum from a patient giving a severe reaction to sulfadiazine was injected into normal skin in four sites, each with 0.1 cc. of the undiluted serum, and on the opposite side of the subject's back five sites, one of undiluted serum, one each of serum diluted 1:10, 1:100, 1:1,000 and 1:10,000. All of these sites in the dilution series were tested with 1 per cent sodium sulfadiazine, which was the soluble form of the drug which had produced the reaction. Even 0.1 cc. of a 1:1,000 dilution of the serum produced a definite sensitization. In order to test the specificity of the reaction the other four sites made with undiluted serum were tested with sulfadiazine, sulfapyridine, sulfathiazols and sulfanilamide, respectively. Sites tested with the first three drugs gave definite reactions; that tested with sulfanilamide showed no reaction. Control tests in non-sensitized skin with the same four drugs were entirely negative. This demonstration of circulating antibody for sulfonamide drugs is relatively rare and not a practical method of determining sensitivity.

In addition to these anaphylactic types of reactions one also sees reactions that show all of the features of serum sickness, and in producing this type of reaction penicillin is by far the commonest drug. Such cases show edema of the face, swelling of the hands and feet with pains in the small joints, fever and intense and often confluent urticaria of the body surface. This type of reaction to penicillin usually has approximately the same incubation period as serum sickness, usually seven to ten days, but occasionally two to three weeks, and follows much the same course. Ordinarily these reactions resolve spontaneously in about ten days or two weeks. Occasionally for inexplicable reasons they may persist for many weeks or months.

Somewhat less similar to serum sickness but still showing the same characteristic incubation period and quite comparable is simple drug fever which may or may not be associated with a skin rash. The skin rash with most drugs is apt to be of the maculopapular type rather than the urticarial type seen with penicillin but may assume various different morphologic appearances. In general these reactions subside as quickly as the drug is excreted. In the case of the sulfonamides, this is a matter usually of only two or three days.

For example, let us consider a febrile reaction to sulfadiazine in a patient with lobar pneumonia making a good initial response to the drug. On the sixth day of treatment there was an abrupt rise in temperature to 104.8°F. at which time the leukocyte count, which had been

7,200, rose to 12,000. The following day it was 26,900 without the presence of eosinophilia. The patient showed no sign of spread or infective complication of the pneumonia. The sulfadiazine was discontinued and in the course of two days the temperature returned to normal and the white count also became normal. In order to prove that this was a reaction to the drug the patient was given small doses of the drug on two successive days and there was a prompt return of a temperature rise, almost as high as before. This illustrates the fact that during the initial course of treatment in the previously non-sensitized patient there is an incubation period of approximately one week before sensitization develops. However, after the patient has recovered he remains sensitive and subsequent doses produce a very prompt reaction, usually within twelve or twenty-four hours.

If the patient is given one of these drugs for only one or two days, it is perfectly possible to develop sensitization but for the drug to be entirely excreted before fever occurs. In this case it is only the immediate reaction when the drug is next used that calls attention to the sensitization.

Another case illustrates what may occur if the drug is not given continually but is given in intermittent dosage. This was a patient with arteriosclerotic heart disease who was treated with mercuhydrin. After several doses of mercuhydrin, which produced no untoward result, the patient began running an irregular high fever, first every other day, then suddenly skipping to three days. It was noted that all of these spikes in temperature corresponded to the injections of mercuhydrin. Thus the form of fever curve depends entirely upon the manner of giving of the drug.

In the treatment of syphilis with arsenicals it was very commonly noted if the doses were given at weekly intervals that the patient would show a febrile reaction the day after the second dose, the so-called "reaction of the ninth day." This was simply due to the fact that the patient who had tolerated the first dose developed sensitization to the drug during the first week and the following dose produced the reaction.

These febrile reactions to drugs usually subside rather promptly when the patient discontinues the use of the drug and might appear to be more confusing than serious. However, there have been certain pathologic lesions noted in these patients if they died, either as a result of the drug reaction or from some other cause shortly after having the drug reaction. Some of these lesions are quite similar to those occurring in serum sickness.

Dr. Arnold Rich has reported arterial lesions in many patients who had sulfonamide reactions. The relationship of this type of drug reaction to clinical periarteritis nodosa is still obscure; but it is apparent that if a patient with an undiagnosed fever has had courses of different antibiotics and sulfonamides, one must be very careful in interpreting the significance of a biopsy which may show this type of lesion.

This lesion also very commonly involves the small vessels in the myocardium, and may be associated with diffuse infiltration. Figure 4 illustrates a section of myocardium from a patient treated with sulfathiazole, showing edema between the muscle fibers and infiltration of a large number of wandering cells, most of which are rather large cells with an eosinophilic cytoplasm.

A different type of lesion which also has been frequently reported in these cases with drug fever due to sulfonamides is shown in Figure 5. This is a section of liver with a small area of focal necrosis in which there is intense infiltration of wandering cells. This same type of focal necrosis may occur in lungs, lymph nodes and spleen. These pathologic lesions suggest that the drug fever, which seems to be a relatively innocuous phenomenon, is actually a manifestation of a sensitization which involves the important viscera, and that continuation of the drug in the face of drug fever or suppression of the drug fever by any agent is a relatively risky procedure which may lead to further visceral damage.

While many different forms of skin rashes are produced by allergic reactions to various drugs, there are two principal types of skin reaction, both of which are produced by many different drugs. One of these is the ordinary maculopapular dermatitis medicamentosa. The other is contact dermatitis, a vesicular reaction occurring after contact of the drugs with the unbroken skin.

Contact dermatitis may be caused by a great variety of topical applications. A patient developed a reaction to penicillin ointment, which was applied to folliculitis in the bearded area. The skin rash continued to spread. As the rash spread the patient put on more ointment until

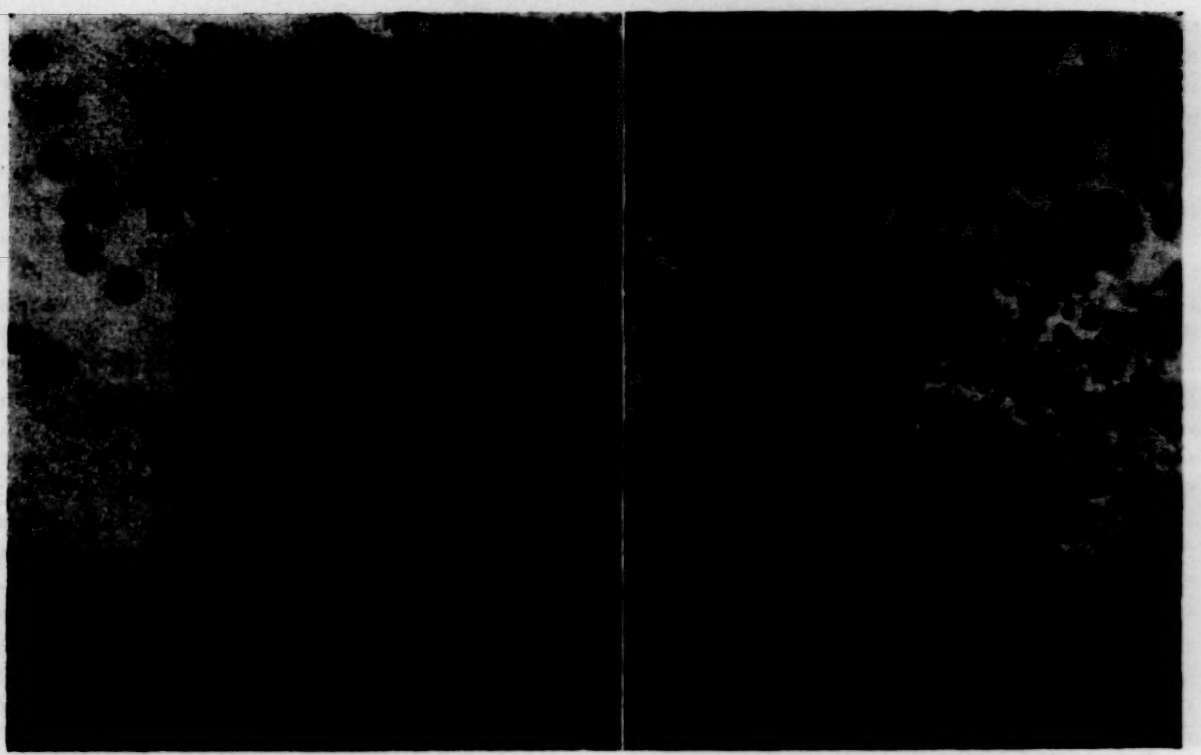

FIG. 4. Infiltration of myocardium due to sulfonamide. (Courtesy of Army Institute of Pathology.)

FIG. 5. Granuloma of liver due to sulfathiazole.

it was soon apparent that he had developed and was constantly spreading a contact dermatitis.

Among the drugs occasionally causing contact dermatitis are the antihistaminic drugs. One patient had an itching eruption on the eyelids for which pyribenzamine ointment was advised. She applied the ointment. The rash spread and only after it had extended down to the chest did she realize that she had something different from the original condition. Applying the ointment and pyribenzamine powder in patches to an uninvolved area of the skin reproduced the rash at that site.

While the vesicular eruption of contact dermatitis and the maculopapular dermatitis medicamentosa are quite different in appearance, actually they represent a very similar type of sensitization. It is common for patients who have had one of these forms of drug sensitization to react to the same drug by the other type of reaction. For example, a patient with ulcers on the legs treated with sulfathiazole ointment developed contact dermatitis due to the ointment. This cleared and a year or more later she was treated with sulfathiazole for pneumonia. When sulfathiazole was given orally, there was a very prompt redness and inflammation at the site of the old contact dermatitis showing that it was the most sensitized part of the skin, but at the same time there was a typical maculopapular rash involving the other areas of the skin. Also in this case there was fever, showing that what appears to be simply a skin sensitization is actually a systemic sensitization. When patch tests of the sulfathiazole were applied to the normal areas of skin, they produced a typical vesicular contact dermatitis.

Time does not permit discussion of less common types of drug dermatitis but there is one that is of special interest in that it shows features similar to the lesions that we have seen in the viscera. This is erythema nodosa. Several types of drugs, such as the sulfonamides and thiouracil, may cause indurated lesions on the legs, indistinguishable in external appearance from erythema nodosa occurring in rheumatic fever or in tuberculosis. In histologic sections of this type of lesion there is seen an intense infiltration of the subcutaneous tissue and perivascular lesions quite similar to those that have been noted in the heart muscle and the liver of patients with drug fever.

There are a great many types of drug reactions that are of great importance and interest,

such as hepatitis due to drug sensitization, agranulocytosis and thrombocytopenic purpura, which cannot be discussed in the time available.

One point that I think should be mentioned is the fact that the specificity of these drug reactions varies a great deal. This is important if a patient has had a reaction to one drug and is to receive another drug related chemically. This has been studied particularly in the case of the sulfonamide drugs and it has been shown that all variations may occur. Thus there may be a specific sensitization to one drug in which the other drugs of the group are easily tolerated; or there may be sensitization to two or three of the group, as in the case mentioned in which the patient was sensitive to sulfadiazine, sulfathiazole and sulfapyridine but strangely not to sulfanilamide, which is the common radical in all of these drugs. Also there have been patients reported who reacted to the entire group of sulfonamides and also to procaine and other compounds which resemble the sulfonamides in having a p-amino benzene group.

STUDENT: Is there any way of preventing serum sickness or of reducing its incidence?

DR. SEEGAL: Serum sickness is more frequent following the injection of large amounts of serum. Purified antibody preparations contain little else than antibody globulin. Hence the same therapeutic dose given as purified antibody globulin has much less total protein and will not give rise to serum sickness as frequently as whole serum. It may be possible to prevent serum shock. The patient should be questioned concerning sensitivity to horse dander and also should be skin-tested with horse serum. In the presence of either a positive history of sensitivity or a positive skin test, a series of small injections of the serum may be given in an attempt to desensitize the patient.

DR. JOHN VAN B. DEAN: Since the injection of serum may produce such severe reactions, what are the indications for using therapeutic antiserums?

DR. SEEGAL: Serum should be injected only when the need is clearly indicated. Today the most common indications for using therapeutic antiserum are diphtheria infection and suspected tetanus infection in a non-vaccinated individual.

STUDENT: What is the treatment of serum sickness?

DR. SHERMAN: The most effective treatment is ACTH by slow intravenous drip. Intramuscular ACTH and cortisone are also effective but less rapid in their action. In the milder cases urticaria may be relieved by antihistaminics and arthralgia by aspirin and codeine.

DR. GEORGE MELCHER: Dr. Sherman, you spoke of the use of skin tests. What are your views on the value of skin tests in allergy?

DR. SHERMAN: The value of skin tests varies greatly in different allergic conditions and with different allergens. Such tests are based on the presumptions that the sensitivity of the skin parallels that of the tissues actually affected in the disease, and that the allergen used is in a form comparable to that which reaches the affected tissue. The former assumption holds true in many common allergic diseases, such as hay fever and asthma due to inhaled allergens, but not in localized allergic reactions such as fixed drug eruptions. The second assumption depends on the nature of the allergen, the changes it undergoes in the body and the method of contact. For example, in bakers' asthma due to inhalation of flour, skin tests with wheat give reliable results, while in asthma believed to be due to eating wheat products their significance is relatively less, since the wheat antigen is cooked, digested and partially metabolized before it reaches the bronchial tissues. In regard to the conditions discussed, skin tests for serum sensitivity are of tremendous value, while in most allergies to non-protein drugs scratch or intracutaneous tests are unreliable and often dangerous. As previously mentioned, patch tests are frequently helpful in the diagnosis of skin eruptions due to drugs, although in the case of rashes from drugs taken internally the patch test reaction occurs only if the drug is also absorbed through the unbroken skin.

DR. JOHN A. COSS: What is the mechanism of the effect of cortisone on allergic reactions?

DR. SHERMAN: The mechanism is not known. During effective control of allergic disease by cortisone the reaction to skin testing with the antigen is not appreciably changed, nor is the concentration of circulating antibody, the reaction in skin sites passively sensitized with a fixed amount of antibody, or the reaction of the skin to injected histamine. One can only presume that the mechanisms may be physiologic rather than immunologic.

DR. EDWARD E. FISHEL: Can penicillin O be used safely in patients who have previously had allergic reactions to penicillin G?

DR. SHERMAN: The available figures show that such patients are less apt to react to penicillin O than to further use of penicillin G. However, penicillin O produces a higher incidence of reactions in patients known to be sensitive to penicillin G than in persons who have not previously received any form of penicillin. If a patient has once had a severe reaction to penicillin G, the safest course is to treat subsequent infections with a completely different antibiotic; but if the organism is presumed to be susceptible only to penicillin, the use of penicillin O would be preferable to re-use of penicillin G.

SUMMARY

DR. GILBERT H. MUDGE: In this clinic we have considered those syndromes or diseases which may be attributed to an antigen-antibody reaction, and especially those instances in which a specific antigen has been administered to a patient. The biologic mechanisms of these reactions have been examined in detail.

Studies in experimental hypersensitivity have emphasized the difficulties inherent in attempts to analyze the clinical counterparts by precise quantitative methods. Thus experimental anaphylactic shock may be produced by the administration of quantities of antigen which give rise to such small tissue concentrations that their presence in tissues can probably not be detected by any known chemical method. The problem of non-precipitable antibodies is also discussed; i.e., antibodies which can not be detected by the conventional precipitin reactions but which are nevertheless active in producing sensitivity. Certain antibodies also show a high degree of specificity with respect to the type of allergic response which they produce.

Serum disease is the classical example of a delayed antigen-antibody reaction. In a previously sensitive patient the administration of a foreign serum, usually horse serum, may produce an immediate anaphylactic response; the more common type is the delayed reaction in a non-sensitive subject. This appears when the antigen is disappearing from the circulation and antibodies to it are beginning to appear. Although the symptoms are often regarded as merely annoying, the possibility must be kept in mind that significant visceral damage may occur in some cases.

In carefully controlled studies the delayed type of skin sensitivity has been used to examine the mechanisms of drug reactions. Derivative antigens are probably always formed; they may be regarded as a complex between the host's protein and the reactive groups of the administered chemical compound. In this type of hypersensitivity the living white blood cell appears to play a unique role. Certain of these mechanisms are presented in detail.

Clinical examples of immediate and delayed types of reactions are given. Although the use of serum has greatly decreased, reactions very similar to serum sickness are frequently seen with such drugs as penicillin. The variable clinical picture of drug reactions is at times difficult to understand. However, the route and frequency of drug administration may be of primary importance in determining the precise clinical syndrome. Variations in drug specificity are illustrated by the sulfonamides; in this group all degrees and patterns of cross reactions have been observed.

Clinico-pathologic Conference

Acromegaly, Mandibular Tumor and Pyrexia

STENOGRAPHIC reports, edited by Robert J. Glaser, M.D. and David E. Smith, M.D., of weekly clinico-pathologic conferences held in the Barnes Hospital, are published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Internal Medicine and Pathology of the Washington University School of Medicine and by Junior and Senior medical students.

THE patient, G. W. (No. 207967), was a Negro housewife, forty-one years of age, who entered the Barnes Hospital for the first time on March 28, 1952, because of a tumor of the jaw. The family history was noncontributory. The past history was of interest in that the patient stated that she had had large hands and feet and unusually prominent facial features for as long as she could remember. Four years before admission frontal and occipital headaches developed and the patient consulted a physician who told her that she had hypertension. She was given sedatives and her blood pressure was said to have fallen to lower levels. Concomitantly the headaches disappeared. The patient had never been intolerant to heat nor had she noted a tremor, but she had become much more nervous than she had ever been before and was easily upset. Her weight had been constant and her menses had been regular until one year before entry when menstrual flow became scanty. In the three months prior to admission she had had no menstrual bleeding. She had never been pregnant. Her voice had always been high pitched. For several years she took large amounts of water and had noted nocturia two times a night.

Four years before entry the patient underwent the first of a series of dental extractions because of carious teeth; all of the involved teeth were removed over a two-year period. Two years before admission to the hospital the patient noted a small nodule at one of the operative sites in the right lower jaw. The nodule increased in size and made it impossible for the patient to wear her denture. With increase in size the lesion also became painful, and the patient came to the Barnes Hospital.

At the time of entry physical examination revealed her temperature to be 36.5°c., pulse 86, respirations 14 and blood pressure 160/100. The patient was a middle-aged Negress with a typical acromegalic appearance. There was marked increase in the size of her hands, feet, nose, lower jaw and ears. The torso was masculine in configuration; the breasts were small. The eyes were prominent but there was neither exophthalmos nor lid lag. Examination of the fundi revealed them to be normal. In the right lower jaw there was a very hard mass measuring 2 by 3 by 3 cm. The mass was fixed to the mandible and was somewhat tender. The patient's tongue was large. A firm mass measuring 2 by 2 cm. was palpated in the left lobe of the thyroid gland. Examination of the heart and lungs revealed no abnormalities. The liver edge was palpable 2 cm. below the right costal margin but the spleen could not be felt. The remainder of the physical examination and the neurologic examination were within normal limits.

The laboratory data were as follows: Blood count: red cells, 4,700,000; white cells, 7,000; hemoglobin, 13.5 gm.; differential count: eosinophiles, 3 per cent; segmented forms, 76 per cent; lymphocytes, 16 per cent, monocytes, 5 per cent. Urine: specific gravity, 1.035; reaction, 6.5; albumin, 1+; sugar, 4+; acetone, negative; sediment: occasional white blood cell per high power field. Blood cardiolipin test was negative. Blood chemistry: non-protein nitrogen, 16 mg. per cent; fasting blood sugar, 171 mg. per cent; sodium, 131.8 mEq./L.; potassium, 4.5 mEq./L; chlorides, 107 mEq./L.; total protein, 8.9 gm. per cent; albumin, 5.3 gm. per cent; globulin, 3.6 mg. per cent; calcium 11.4 mg. per cent; phosphorus, 4.6 mg. per cent; alkaline phosphatase, 5 Bodansky units. Protein-bound iodine, 7.3 gamma per cent. Radioiodine studies: uptake, 22.4 per cent, excretion, 56 per cent. Roentgenograms: the mandible was edentulous, enlarged, heavy and sclerotic. In the anterior portion of the right body there was an oval mass of irregularly sclerotic bone with a rim of radiolucency suggesting a fibrous osteoma. A

similar small process involved the alveolar ridge of the left maxilla. There were degenerative and sclerotic changes in the cervical spine, and variation of the shapes of the vertebrae with marked spur formation and irregularity of the superior and inferior cortical margins suggestive of old osteochondritis. The heart was minimally enlarged. The lungs were clear. The calvarium was diffusely thickened; the outer table was densely sclerotic with prominences in the region of the bregma and the occipital protuberance; there was also thickening of the internal table, especially in the frontal region. The sella turcica was enlarged with good cortical margins; no erosion or demineralization was noted. Some widening of the prevertebral soft tissue was seen suggestive of possible extension of the tumor through the base of the sella. Electrocardiogram showed left ventricular enlargement; myocardial ischemia.

On a regular diet the patient spilled between 3 and 33.5 gm. of glucose daily. She was then given a diet containing 200 gm. of carbohydrate, 100 gm. of protein and 90 gm. of fat. On this dietary regimen she showed only traces of glucose in the urine. On the fourteenth hospital day, after various studies were completed, the patient underwent operation for the removal of the tumor mass in the mandible. She was given sodium pentothal, nitrous oxide-oxygen anesthesia and the lesion was excised. Iodoform and balsam of Peru packs were inserted into the operative wound. Prior to operation the patient was begun on prophylactic penicillin, 400,000 units twice daily, but during the evening after operation, her temperature rose to 38°C. and on the following day reached 39°C. A repeat chest x-ray showed only slight atelectasis on the right. The patient's urinary output was rather scanty on the first two postoperative days, but thereafter was satisfactory. On the third postoperative day her temperature was 39.5°C. No apparent response to penicillin and streptomycin therapy was noted. She complained only of tenderness along the right lower jaw and of some difficulty in flexing her neck. The difficulty in flexion of the neck was thought to be due to pain in the jaw and to the fact that her neck was short. The operative wound appeared clean and the visual fields remained normal. A repeat electrocardiogram showed no significant change from the one obtained prior to operation. Physical examination showed no significant change; the lungs remained clear except for occasional scattered rales in the right base which cleared on coughing. There was no tachycardia. The white blood count was 8,500; urine and blood cultures were negative. On the evening of the third hospital day penicillin and streptomycin were discontinued and aureomycin therapy was instituted. On the fourth postoperative day the serum calcium and phosphorus levels were repeated and found to be normal. No signs of infection at the operative site or elsewhere were noted. The patient's temperature rose to 40.6°C. on the fifth postoperative day, and because she continued to complain of difficulty in flexing the neck, a lumbar puncture was performed. The initial pressure was 300 and the final pressure 220 mm. of water. The fluid contained sixty-six cells without acid and twenty-four cells with acid; 95 per cent of the cells were lymphocytes. The protein was 57 mg. per cent.

On the sixth postoperative day the patient's temperature fell to normal and she appeared improved. The following day, however, her temperature again rose, and on the eighth day after operation reached 40.4°C. A second lumbar puncture was performed; the initial pressure was 200 and the final pressure 180 mm. of water. There were twenty cells with acid of which 60 per cent were lymphocytes. The spinal fluid sugar was 44 mg. per cent. Cultures of the fluid including those for tubercle bacilli and for fungi were negative. Despite the fact that no specific diagnosis had been made, the patient was given aspirin in an attempt to lower her temperature. There was a moderate decrease in fever for several days but on the ninth postoperative day, when the temperature rose to 40.2°C., there was no response either to large amounts of aspirin or to continuous sponging. The patient was kept in an oxygen tent. During the entire postoperative period her blood pressure fluctuated somewhat but averaged about 120/80. Repeated determinations of the serum electrolytes and of the white blood count were normal. Late on the ninth postoperative day the patient suddenly expired. Rectal temperature taken fifteen minutes after death was 41.6°C.

CLINICAL DISCUSSION

DR. CARL V. MOORE: It is clear that this forty-one year old Negress was suffering from classic acromegaly. She had done quite well except for polydipsia and polyuria, neither of which was especially troublesome. She came to the dental clinic because of the painful nodule

in her lower jaw which made it impossible for her to wear a denture. She was admitted to the surgical service for removal of the lesion but prior to operation was transferred to the Medical Service where the studies described in the protocol were performed. Dr. Wilson, from the description of the roentgenologic findings, I assume this patient exhibited many of the characteristic changes seen in acromegaly.

DR. HUGH M. WILSON: Yes, she did. The mandible and the sella turcica were the sites of typical alteration.

DR. C. V. MOORE: Were the x-ray findings of the mass in the mandible suggestive of a specific type of lesion?

DR. WILSON: We thought the tumor was a fibrous osteoma.

DR. C. V. MOORE: One of the first questions which was raised when this patient was admitted to the hospital was whether the lesion in her jaw had any relationship to the pituitary tumor. Dr. Peterson of the Department of Oral Surgery is here, and I would like to ask him to discuss this point.

DR. LEROY W. PETERSON: As far as I know there is no specific relationship between osteomas, such as the one this patient had, and acromegaly. I could find no reference in the literature which suggests that their concomitant occurrence is anything other than a coincidence.

DR. C. V. MOORE: Dr. Moore, you have been interested in this subject for a long time. Do you have any comments to make?

DR. SHERWOOD MOORE: I would agree with Dr. Peterson. I have not been impressed by the occurrence of osteomas in acromegaly either.

DR. C. V. MOORE: I understand, Dr. Waldron, that you have a section of the osteoma which was removed at the time of operation. Would you demonstrate it?

DR. CHARLES WALDRON: Roentgenograms of the mandibular lesion after removal showed clearly that it was a well delimited tumor which appeared to be encapsulated. Figure 1 is typical of all the sections. It shows a mass of dense bone and heavy trabecula between which densely fibrous marrow is seen. I prefer to call this tumor an osteoma for it appears to be an expanding neoplasm of bone but its resemblance to a late stage of fibrous dysplasia, particularly in the microscopic sections, must be admitted.

DR. C. V. MOORE: Do you think the osteoma was related to the pituitary tumor?

DR. WALDRON: No, I do not. I discussed this question with Dr. William H. Bauer of the Department of Pathology at St. Louis University; he has had a vast experience in this particular field and he thinks there is no relationship between the two.

DR. WILLIAM H. DAUGHADAY: In reviewing case reports of acromegaly I found one case in which multiple osteomas of the skull were present.

DR. C. V. MOORE: When this patient was transferred to the Medical Service, one of the major problems was to decide whether the acromegaly should be treated prior to surgical removal of the osteoma. There was some difference of opinion among those who saw the patient as to whether radiation should be given or whether she should be treated conservatively. It will be recalled that the patient had diabetes with polydipsia and polyuria and an elevated basal metabolic rate (+40) with nervousness. In addition, the eosinophile count was normal; there were no neurologic abnormalities, and as far as could be determined, the physical signs of acromegaly had been static. Finally, there was evidence of increased production of growth hormone; the latter information has not been available in the past, and I would like, therefore, to ask Dr. Recant to comment on this determination and its significance.

DR. LILLIAN RECANT: We have recently observed in the serum of fasted normal patients a respiratory quotient depressing factor. Serum obtained from normal patients not in the fasting state does not contain the factor. On the other hand, in several diabetic patients and in this patient with acromegaly, the respiratory quotient depressing factor was present in significant quantities in serum obtained from them in the fed state. The factor resembles growth hormone in many respects but whether it is identical with growth hormone is not certain.

DR. C. V. MOORE: Dr. Daughaday, would you discuss the problem as to whether a patient with acromagaly should or should not receive treatment for the disease?

DR. DAUGHADAY: In the first place it should be pointed out that the circumstances in this case were rather unusual in that the patient came to the hospital for treatment of an incidental complaint. Most patients with acromegaly seek hospitalization because they have symptoms referable to their primary disease. When patients present with symptoms which are troublesome to them, we usually suggest a course of x-ray

therapy provided there is little restriction of visual fields. In this particular instance, however, I believe I would have suggested deferring treatment until after removal of the osteoma. Radiation therapy usually requires a period of three to six months before improvement appears and symptoms referable to treatment may develop during this period. This woman was actually in remarkably good condition despite the fact that she had some symptoms of diabetes.

DR. C. V. MOORE: Do you believe that her tumor was still functioning or do you consider that it may have been "burned out"?

DR. DAUGHADAY: Until there is a satisfactory method for determining hormone titers in the blood, the answer to that question will have to be based on clinical impressions only. This patient apparently had increased growth hormone production although the evidence for this statement is indirect. The normal eosinophile count is not particularly helpful since it quite commonly is normal in actively progressive acromegaly. Similarly, many patients with acromegaly may have no neurologic findings because there may be even less encroachment on the sella turcica than this patient exhibited. The fact that the physical signs were fairly static is difficult to evaluate. Mention should be made of the serum phosphorus level as a means of determining activity in acromegaly. Reifenstein and Albright suggest that when the serum phosphorus level is above 4.5 mg per cent, activity is probable. Taking all the data together I would guess that this woman's disease was still active although not markedly so.

DR. C. V. MOORE: In his article on hyperpituitarism in Oxford Medicine, Dr. Harry B. Friedgood has this to say: "The ideal patient for roentgen therapy is one who has endocrine disturbances and possibly a slight enlargement of the sella turcica but no visual disorders." This patient then, according to Dr. Friedgood's views, would have been a good candidate for roentgen therapy. As far as we know no final decision was made as to whether the patient was to be treated after the removal of the tumor or not. I would like to ask Dr. Schwartz whether patients with active acromegaly are endangered if they are subjected to surgery elsewhere in the body before therapy is given to the pituitary tumor.

DR. HENRY G. SCHWARTZ: No, I do not think they are; I see no reason why the osteoma should not have been removed first.

DR. C. V. MOORE: If patients present visual disturbances, should radiation be given?

DR. SCHWARTZ: In my opinion the presence of visual disturbances makes it mandatory that the patient be subjected to craniotomy.

DR. C. V. MOORE: Dr. Park, this patient was on your service when she entered the hospital. You favored deferring radiation therapy, did you not?

DR. CHARLES R. PARK: Yes, I did.

DR. C. V. MOORE: Dr. Wood, what was your opinion?

DR. W. BARRY WOOD, JR.: I thought that the patient should have the osteoma removed first, and that subsequently she should receive radiation. It seemed to me that radiation as a prophylactic measure was justifiable since it might prevent the patient from getting into serious difficulties later. One of the major problems we considered was whether or not she had hyperthyroidism. There were a number of features which suggested it, in addition to the high basal metabolic rate.

DR. C. V. MOORE: The diagnosis of hyperthyroidism came up for discussion during the postoperative course also, Dr. Wood, when the possibility as to whether the patient may not have had a thyroid storm was considered. Dr. Daughaday, do you think this woman had thyrotoxicosis?

DR. DAUGHADAY: About 50 per cent of patients with acromegaly exhibit an elevated basal metabolic rate. Cushing found, however, that the thyroid gland from most patients with acromegaly was not obviously hyperplastic when examined microscopically but rather that the nodular enlargement was due to follicles distended with colloid; the epithelium was flat. McCullagh, Gold and McKendry[1] performed radioactive iodine uptake studies on seven patients with acromegaly and found normal values in all of them, despite the fact that each patient had an elevated basal metabolic rate. Further, protein-bound iodine has not been elevated in most patients with acromegaly. The evidence, therefore, suggests that acromegaly may lead to an elevated basal metabolic rate without hyperthyroidism *per se*. I doubt that this woman had significant hyperthyroidism.

DR. C. V. MOORE: Let us now consider the

[1] MCCULLAGH, E. P., GOLD, A. and MCKENDRY, J. B. R. Radioactive iodine uptake in the hypermetabolism of acromegaly. *J. Clin. Endocrinol.*, 10: 687, 1950.

postoperative course further. It will be recalled that on the night of operation, fever developed and the patient's temperature rose rapidly, despite the fact that she had been begun on antibiotic therapy prior to operation. On the third day stiffness of the neck developed; this complaint was thought to be referable to pain in the operative site and to the fact that the patient's neck was very short. At the time the first lumbar puncture was done, the spinal fluid pressure was elevated and there were twenty-four cells with acid. The patient continued to have fever which rose to a very high level terminally, but during the entire period she remained alert. There is a note in the chart stating that she talked coherently with the nurses and house officers half an hour before she expired. The cause of the postoperative febrile course was a very difficult one to determine. One of the obvious considerations was the possibility of pulmonary infection. Dr. Wilson, were there any findings in the postoperative chest film which would be helpful in this regard?

Dr. Wilson: Minimal cardiac enlargement was again demonstrated. In addition, the diaphragms were elevated but no distinct areas of pulmonary infiltration were noted.

Dr. C. V. Moore: Dr. Hunter, will you open the discussion of the postoperative course.

Dr. Thomas H. Hunter: When Dr. Paul B. Beeson was visiting here, he saw this patient and presented a thorough exposition of the possible causes of her fever. I have had the advantage, therefore, of Dr. Beeson's thoughts in the case. Perhaps the most impressive findings were those related to the spinal fluid. The patient had both elevation in pressure and increase in cells. Although the pleocytosis was not marked, it was nonetheless definite. Until the normal value for the spinal fluid sugar was obtained, tuberculous meningitis, among other things, merited serious consideration and brain abscess was also a possibility. Neither could be substantiated. A central lesion of some type seems most probable.

Dr. Henry A. Schroeder: I think one can localize further the central nervous system involvement in this instance. Lesions in the posterior hypothalamus characteristically produce hypothermia whereas those in the anterior hypothalamus are usually associated with hyperthermia. I would suggest, therefore, a lesion in the anterior hypothalamus.

Dr. C. V. Moore: A central lesion was seriously considered by the staff caring for the patient. Do you have any comments about this possibility, Dr. Schwartz?

Dr. Schwartz: Dr. Schroeder has intentionally oversimplified the relationship of hypothalamic lesions to hypothermia and hyperthermia, but his generalization is a very satisfactory one for the purposes of this discussion. Since this woman almost certainly had a pituitary tumor of the eosinophilic type, it would have been entirely possible for the tumor to have extended through the sella, and thus to have involved either the anterior or posterior hypothalamus. Lateral extension would have given rise to temporal lobe signs.

Dr. C. V. Moore: If such a complication did occur, do you think it was related to the surgical procedure performed twelve hours earlier?

Dr. Schwartz: I doubt that the operation had anything to do with it. In acromegaly a course such as this patient exhibited is quite common; indeed, just such a course may occur if the patient has had neither x-ray therapy nor an incidental operation, or conversely, if either of the latter two has transpired.

Dr. C. V. Moore: Does the occurrence of hyperpyrexia in a patient with acromegaly constitute an indication for craniotomy?

Dr. Schwartz: No, I do not think so. When such a situation develops, one must go through the differential diagnosis of pyrexia, as was done in this case; meningitis is an important possibility. It must be remembered that antibiotic therapy may modify the clinical picture of meningitis without completely controlling the infection, and it may be difficult, therefore, to rule out meningitis. In regard to extension of the pituitary tumor *per se* an important point against this complication in the present patient was the absence of hypotension and other signs of adrenal insufficiency which are common under such circumstances.

Dr. C. V. Moore: In summary, the consensus appears to be that the osteoma had nothing to do with the acromegaly, and that its removal probably did not in itself significantly influence the patient's course. It seems likely that the postoperative fever was due to a central lesion, possibly a hemorrhage into the gland itself. Are you satisfied, Dr. Wood, that the patient did not have hyperthyroidism?

Dr. Wood: Yes, I would agree with Dr. Daughaday in that regard.

Dr. C. V. Moore: Are there any other comments?

DR. SCHWARTZ: I should like to emphasize that in the studies made by Hausner and Adams,[2] there was no evidence that operative intervention elsewhere in the body played a role in pituitary necrosis or hemorrhage. As suggested previously necrosis and hemorrhage occur in patients with acromegaly, both in those given x-ray treatment and in those untreated.

Clinical Diagnoses: Acromegaly; pituitary hemorrhage or necrosis?

PATHOLOGIC DISCUSSION

DR. WILLIAM R. MURPHY: In addition to the external features of acromegaly described clinically the axillary, epitrochlear and inguinal lymph nodes were enlarged and the skin was greatly thickened and extremely tough. The skull was quite thick and dense, especially in the frontal region where it measured 20 mm. in thickness. The brain showed no hemorrhages, petechiae or other lesions of the hypothalamus or other regions. The diagraphma sellae was bulging to approximately ½ cm. above its usual position and pressed on the floor of the third ventricle. The pituitary weighed 6.1 gm., which is ten times the normal weight. On cross section the superior portion of the pituitary and pituitary stalk had the dark red color of fresh hemorrhage; the remainder was very friable grey tissue.

The nodular thyroid was enlarged twofold and contained obvious glassy brown colloid. A grey-brown parathyroid adenoma 1 cm. in diameter was found imbedded in the lower portion of the right lobe of the thyroid. Another parathyroid 3 mm. in diameter and of similar color was found posterior to the left lobe of the thyroid. There was generalized splanchnomegaly. The liver weighed 2,000 gm. and the kidneys and spleen were moderately enlarged. The adrenals were not remarkable. Slight congestion and edema and a few focal subpleural hemorrhages in the lungs were the only significant lesions in the thoracic viscera. There were strong fibrous adhesions in the pelvis and the ovaries were adherent to the posterior surface of the bladder.

DR. DAVID E. SMITH: Most of the questions that have been discussed clinically were answered by the gross findings. The cellular nature of the tumor and anatomic evidence of its recent activity are interesting points that may be answered by histologic examination. Figure 2 illustrates a section from the pituitary. It is a picture of necrotic tissue with interstitial hemorrhage. At the periphery are a few intact but pyknotic cells. Special stains did not demonstrate specific granules in any cells in these sections. The recent necrosis and hemorrhage into the tumor probably eliminated specifically staining substances by destroying the cells; however, the possibility remains that some of the intact cells represent "burned out" acidophiles. This hypothesis of degranulation by exhaustion is an attractive explanation of those cases of acromegaly that begin to show manifestations of hypopituitarism, but histologic proof of such a phenomenon is usually complicated as in this case by old or recent necrosis in the adenoma. We cannot do much more than postulate the answer to such a question in the present case, but certain other tissues might be indicative of the amount of recent hormonal activity of the tumor.

The thyroid in Figure 3 shows one of the nodules which was recognized grossly. The acini are filled with colloid, and the epithelium is of a low cuboidal character. This thyroid shows no anatomic evidence of cellular hyperplasia, but rather is in a stage of nodular colloid hyperplasia. Such a histologic picture so common in acromegaly might be interpreted as evidence of a previous and probably intermittent activity of a thyrotrophic hormone. Such a concept is in harmony with a popular theory of the evolution of nodular colloid goiters. Although it is far from unanimously agreed that the acidophilic cells elaborate thyrotrophic hormone, some investigators have found in the material removed at operation from eosinophilic adenomas of the pituitary evidence of a substance with a stimulating effect on the thyroid glands of experimental animals.

The parathyroids in this case are also very interesting. Although it is not generally recognized that the pituitary secretes a hormone that acts on the parathyroids, it is tempting to consider the adenomas found here as evidence of such a substance. There is no evidence of the usual changes of hyperparathyroidism elsewhere in these tissues, but the effect of the manifest disorder of bone growth on these glands can

[2] BROUGHAM, M., HEUSNER, A. P. and ADAMS, R. D. Acute degenerative changes in adenomas of the pituitary body—with special reference to pituitary apoplexy. *J. Neurosurg.*, 7: 421, 1950.

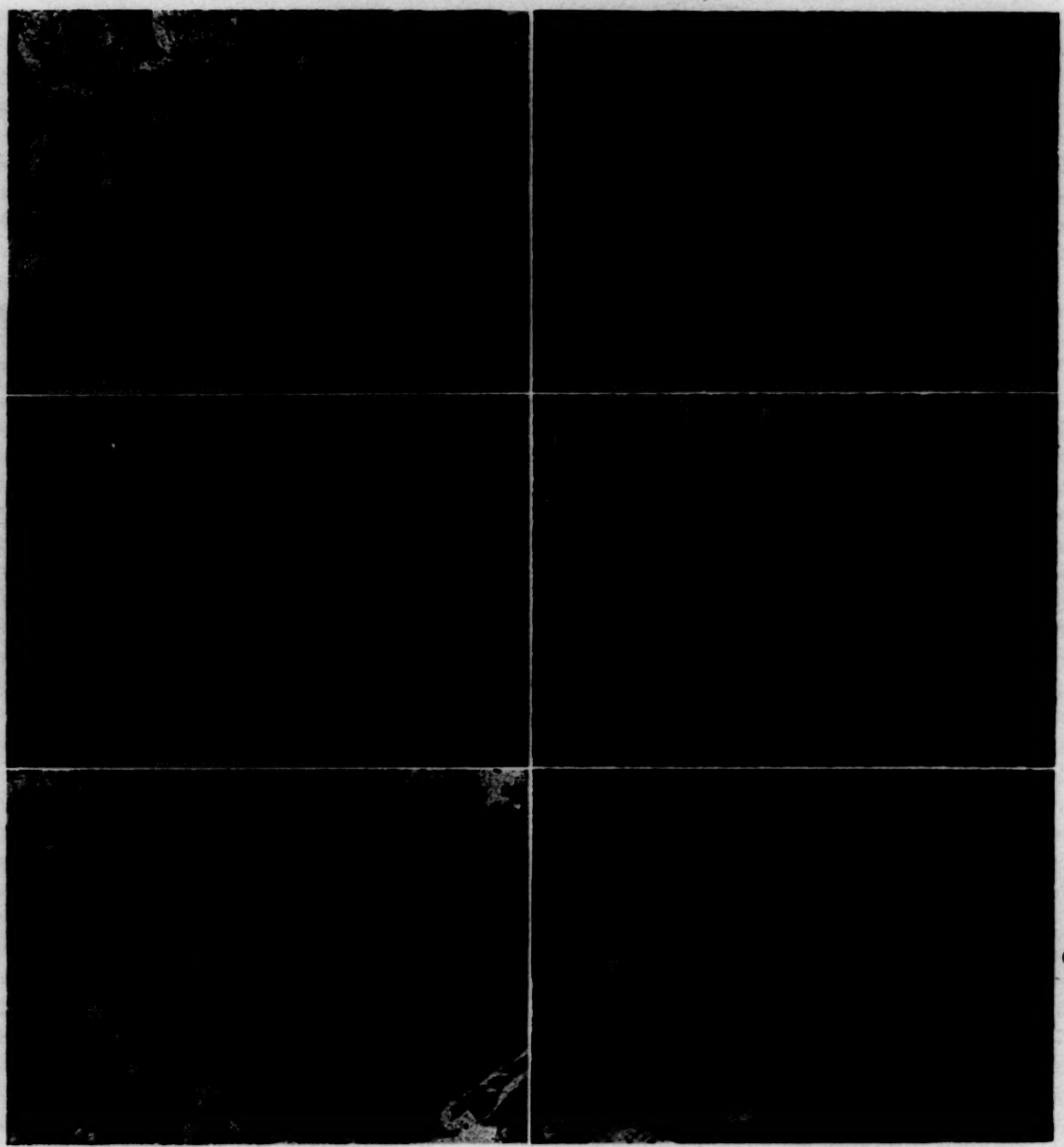

FIG. 1. A section of the mandibular osteoma. Characteristic features are the heavy trabecula, large areas of dense bone and fibrosis of the marrow. The well delimited outline of this tumor was best appreciated in the roentgenograms.

FIG. 2. The adenoma of the pituitary showing almost total necrosis of the cells and interstitial hemorrhage.

FIG. 3. Thyroid with a typical nodular colloid hyperplasia.

FIG. 4. Adenoma of the parathyroid. Its mixed character is indicated by the oxyphiles in the upper right corner and the chief cells in the remainder of the section. No evidence of hyperparathyroidism was found.

FIG. 5. Dense cortical bone that formed the thickened and confluent diploe of the skull.

FIG. 6. A costochondral junction showing thickened trabecula in the marrow cavity and no active bone formation to suggest progression of the acromegaly.

not be fully assessed in the light of present knowledge. The histologic picture as shown in Figure 4 is one of a mixed cell type with nodules of oxyphil cells as in the upper right corner and a predominance of chief cells in the remainder of the adenoma.

The section of the skull illustrated in Figure 5 is almost uniform cortical bone. There are only very small marrow cavities and no evidence of especially active resorption or formation of bone. We can only offer this as a histologic demonstration of an unusual phenomenon. Neither anatomic nor physiologic evidence presently enables us to explain it. In Figure 6 the cancellous bone beneath a costochondral junction shows a degree of the same thickening. This photomicrograph also adds to the evidence that the pituitary tumor had not been active for some time, for characteristically in acromegaly there is a resumption of bone formation at epiphyses that are not completely ossified, such as the costochondral junctions. No such activity is apparent here. This phenomenon accounts for the characteristic overgrowth of the jaw, for the epiphysis of the mandibular condyle normally does not close as do those of the bones of the extremities.

In reviewing this case the diabetes is the best evidence that there was recent metabolic activity on the part of the tumor of the pituitary. The adrenals, for instance, were quite small. This is, therefore, a case of acromegaly due undoubtedly to an acidophilic adenoma of the pituitary, but it is not possible to confirm its acidophilic nature or directly evaluate its present activity because of the hemorrhagic necrosis. The etiology of hemorrhagic necrosis in adenomas of the pituitary is unknown. It is possible in this case that mechanical force transmitted directly up the mandible from the operative site might have played a part in disrupting an inherently abnormal vascular bed in the tumor. The only anatomic finding that can be related to the cause of the hyperthermia is the bulging of the diaphragm of the sella turcica caused by the acute hemorrhage and necrosis in the tumor. This definitely pressed upon the floor of the third ventricle and hypothalamus, but no acute lesions were discernible in the latter structure to gross inspection or in microscopic sections.

Final Anatomic Diagnoses: Adenoma of the pituitary; acromegaly; necrosis and interstitial hemorrhage in the adenoma of the pituitary.

Acknowledgment: Illustrations were made by the Department of Illustration, Washington University School of Medicine.

Case Reports

Thrombotic Thrombocytopenic Purpura*

Confirmation of Clinical Diagnosis by Bone Marrow Aspiration

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SINCE the original description by Moschcowitz in 1925[1] thirty-eight instances of an unusual disorder characterized pathologically by the presence of widely disseminated thrombotic occlusions of terminal arteries, arterioles and capillaries have been recorded.[2–24] While there is now evidence to the contrary, most early observers considered that the thrombi were composed of platelets or disintegrated platelet material.

The common clinical features accompanying this pathologic curiosity have been clearly delineated. Suspicion should be aroused by the association of hemolytic anemia and thrombocytopenic purpura with an illness which is febrile and often fulminating. The entity is further distinguished by evidence of involvement of the central nervous system which may be transient or progressive in character. All reported cases have terminated fatally, usually within a few weeks following onset of symptoms. The characteristic syndrome has appeared in some instances to be a terminal event of a more extended illness.[2,7,20,21]

Clinical investigation has been hampered because the correct diagnosis has rarely been definitely established during life in the few instances in which it has been strongly suspected.

Singer and associates[19] carefully studied the hematologic and serologic aspects of a single case but were unable to demonstrate a pattern which was diagnostically specific. Beigelman[20] reported an atypical case in which platelet thrombi were noted at biopsy of the skin of the patient. The significance of this finding was apparently not appreciated, however, until necropsy. Meacham and co-workers[21] have recently reported that in the study of a patient with prolonged disease, retrospective examination of sections of the spleen (removed three years previously because of hemolytic anemia) disclosed multiple arteriolar thrombi. Studies at necropsy demonstrated disseminated vascular lesions compatible with the diagnosis of thrombotic thrombocytopenic purpura. Muscle biopsy in the case recently reported by Blackman, Cohen and Watson[24] was not helpful.

The frequent observation of typical lesions involving vessels of the bone marrow in necropsy material suggested to us that correct diagnosis of this disease might be established before death of the patient by means of aspiration of bone marrow. To our knowledge this has not heretofore been accomplished.

This report presents two cases in which the presumptive clinical diagnosis of thrombotic thrombocytopenic purpura was confirmed by examination of paraffin sections of small fragments of material obtained from the sternal bone marrow by needle aspiration.

REPORT OF CASES

CASE 1. A housewife, twenty-two years of age, was admitted on June 5, 1951, complaining of weakness, fever and hemoptysis. Her illness had apparently begun approximately two months before with a cold. Shortly thereafter she noted low-grade fever and a cough which raised small amounts of sputum which was frequently blood streaked. One month later roentgenographic and laboratory studies by her local physician were said to have given support to diagnoses of virus pneumonia and anemia. Her condition grew steadily worse despite the administration of aureomycin, penicillin and four blood transfusions. Following the last transfusion emesis occurred and the patient was

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unable to lift the right arm for a period of ten minutes.

There was no history of significant familial disease. The patient had been generally well except for a miscarriage followed by uterine dilatation and curettage which occurred in July, 1950. Thereafter she was easily fatigued but otherwise subjectively well until the onset of her current illness.

Examination revealed pallor, lethargy and a poor state of nutrition. There were small ecchymoses at the sites of previous hypodermic injections. The temperature was 99.8°F., the pulse rate 96 beats per minute, the respiratory rate 18 per minute, and the blood pressure expressed in millimeters of mercury was 110 systolic and 60 diastolic. Funduscopic examination revealed a single region of soft exudation above the left optic disk. No retinal hemorrhages were observed. There was slight depression of breath sounds in the right axillary zone. Dullness over the liver on percussion extended 3 cm. below the right costal margin. The tip of the spleen was palpable when the patient inspired deeply. Shotty inguinal lymph nodes were palpable. Aside from softness of the corpus uteri, the pelvic organs were apparently normal. The patient was menstruating. Neurologic examination disclosed nothing remarkable.

Laboratory studies revealed the presence of anemia, the value for hemoglobin being 7.5 gm. per 100 cc. of blood and for erythrocytes 1,800,000 per cu. mm. with 12.7 per cent reticulocytes. The leukocyte count was 8,400 and platelets numbered 32,000 per cu. mm. of blood. Study of stained smears of the blood revealed hypochromasia, polychromasia and numerous normoblasts. There was no microspherocytosis. Toxic changes were present in the neutrophils and there was a shift to the left in the myeloid leukocytes, occasional stem cells being noted.

Tests of the fragility of the erythrocytes, using both heat and acid, gave normal results. Prothrombin times (Quick) ranged from 21 to 24 seconds (normal = 17 to 19 seconds). The bleeding time was prolonged and the Rumpel-Leede phenomenon of minute subcutaneous hemorrhages was easily demonstrated when a tourniquet was applied to the upper arm. A test for clotting time of the plasma after recalcification gave a value of 183 seconds (normal = 60 to 120 seconds).

Results of serologic tests for syphilis were as follows: Kline, 4+; Kahn, 3+; Hinton, positive; and Kolmer, weakly positive. Other tests made on serum obtained from this patient disclosed the following constituents to be present in the indicated titers: complement (measured by use of erythrocytes of sheep), 1:64; heterophile antibody, 1:128; auto-agglutinins, 1:8, iso-agglutinins, 1:16; cold agglutinins, 1:32. Coombs' test gave negative results.

Bromsulfalein test of liver function revealed retention of dye, grade 2. Result of the direct test for serum bilirubin was negative but the value according to the indirect procedure was increased to 2.2 mg. per 100 cc. (normal = 0.6 mg. per 100 cc.). Blood urea measured 30 mg. per 100 cc. Urinalysis gave essentially normal results when the patient first registered, but subsequent specimens of urine contained gross blood.

Results of other studies, including skin tests with tuberculin and histoplasmin, cultures of blood and sputum for various bacteria and fungi, examination of sputum for malignant cells and Friedman test for pregnancy, were all negative.

Roentgenographic examination of the thorax revealed pneumonic consolidation involving the anterior segment of the upper lobe of the right lung and the lateral segment of the middle lobe of the right lung.

Further examination of the patient was carried out in an attempt to exclude all but one of the three tentative diagnoses following: leukopenic leukemia, thrombotic thrombocytopenic purpura and acute disseminated lupus erythematosus.

Aspiration of the sternal bone marrow was performed. The myeloid-erythroid ratio was 1:1.3. Study of films stained by Wright's method revealed hypercellular marrow with normoblastic erythropoiesis and myelopoiesis both of which showed a shift to the left, reticuloendothelial phagocytosis of erythrocytes and pigmented granules. Megakaryocytes appeared normal in number but displayed a moderate shift to the left and little evidence of formation of platelets. Paraffin sections made from the solid marrow particles found in the aspirated material and stained with hematoxylin and eosin revealed hyperplastic marrow. The blood vessels were rendered conspicuous by the presence of extensive endothelial proliferation, dilated segments and finely granular thrombi. (Fig. 1.) In some areas there was fibroblastic infiltration of the thrombi. Study by means of

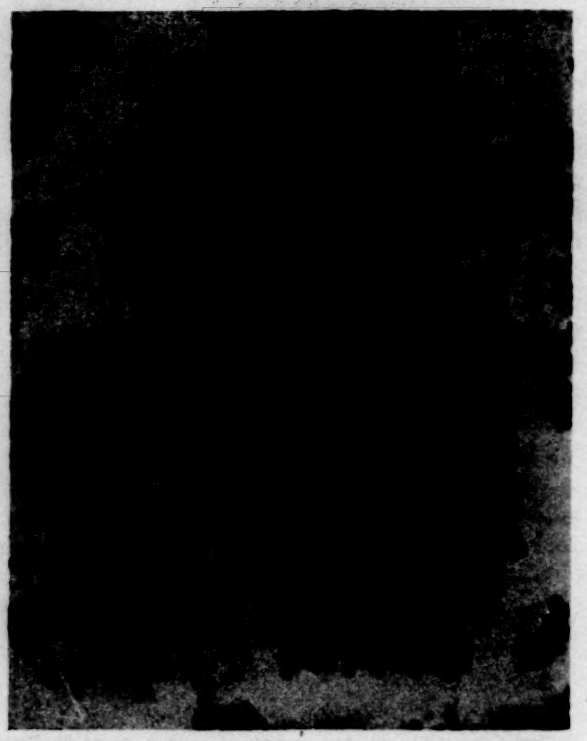

FIG. 1. Case 1. Section of biopsied bone marrow. Note saccular dilatation of blood vessel, endothelial hyperplasia and finely granular thrombotic material with cellular infiltration; hematoxylin and eosin × 250.

special stains was not conclusive as to the nature of the thrombotic material.

The observation of these thrombi, together with the clinical features of the case, established the diagnosis of thrombotic thrombocytopenic purpura. The precise nature of the associated pulmonary lesion remained undetermined.

Because of clinical and pathologic similarities between this syndrome and the collagen disorders, particularly disseminated lupus erythematosus, a therapeutic trial with corticotropin (ACTH) or cortisone seemed worth while. Accordingly cortisone in a dosage of 300 mg. daily was administered during the last three days of life. General supportive measures, including administration of five transfusions of blood of 500 cc. each, were also carried out. There was no apparent change in the fulminating course of the disease nor were the values for hemoglobin or erythrocytes significantly improved following the transfusions.

The patient remained stuporous and disoriented with temperature ranging from 99° to 104.2°F. The skin and sclerae became obviously icteric and gross bleeding from all orifices occurred during the last forty-eight hours. Coma and repeated generalized convulsions developed during the terminal twelve hours of the patient's illness; however, no signs appeared of focal involvement of the central nervous system.

Necropsy was performed one and a half hours after death. The body was emaciated and the skin exhibited a few petechiae and ecchymoses. Petechiae were present in the pleurae, pericardial sac, epicardium, peritoneum and gastrointestinal tract. A tumor mass 18 by 14 by 8 cm. occupied the anterosuperior portion of the mediastinum. This mass extended laterally into the upper and middle lobes of the right lung and the bronchi at the hilus. It surrounded the great vessels without compressing their lumina. The cut surface was yellow-white and firm with numerous hemorrhages scattered throughout. Dense trabeculae traversed the substance of the tumor. Microscopically, it was composed of cells with small pyknotic nuclei and scanty cytoplasm. There were also a few larger cells with vesicular nuclei and more abundant cytoplasm. Much of the tumor mass was necrotic and in other areas the mass was replaced by dense fibrous connective tissue. The tumor involved the regional lymph nodes and the right lung. The histologic character of this tumor was consistent with the diagnosis of malignant thymoma.

The heart weighed 285 gm. The pericardial sac was infiltrated by tumor but the myocardium itself was not involved. Scattered throughout the heart in all its layers were numerous petechiae. Many of the vessels contained thrombi made up of masses of hyalin and some fibrin. These thrombi were in varying stages of development. (Fig. 2*a*.) Some of these thrombi were organized and recanalized. Saccular and fusiform aneurysms of the small arteries and arterioles were common and were demonstrated by serial sections.

The lungs were edematous. Two-thirds of the upper lobe of the right lung was invaded by the mediastinal tumor mass. A nodule 2 cm. in diameter was also present in the upper lobe of the left lung. The lower lobe of the left lung exhibited obstructive pneumonitis. Many of the alveolar arteries contained hyalin masses. Superimposed on the hyalin masses in the wall of these arteries were thrombi containing a few strands of fibrin and an occasional platelet.

The spleen weighed 510 gm. The congested pulp was replaced by infarcts of varying sizes and ages. Both the small and middle-sized

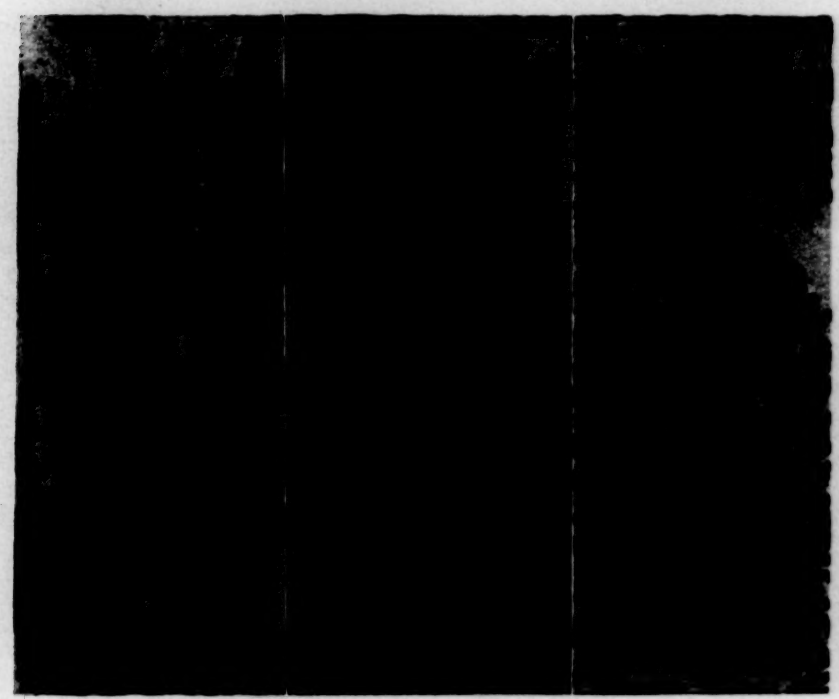

FIG. 2. Case 1. (*a*) Heart; multiple thrombi in arteries of myocardium. (*b*) Kidney; thrombus in renal artery with organization. (*c*) Bone marrow; hyalin body in wall of vessel; note megakaryocytes; all hematoxylin and eosin × 375.

arteries contained hyalin-like masses associated with recent and organizing thrombi. Erythrophagocytosis was rampant. Many megakaryocytes were seen in the spleen. Small foci of myeloid metaplasia were noted.

The enlarged, congested liver contained a few petechiae. Microscopic examination revealed swollen granular liver cells and many of these cells contained an increased amount of bile pigments. The reticuloendothelial cells were increased in number and contained much iron pigment. Only an occasional occluding thrombus was seen in the arteries. The gastrointestinal tract appeared normal except for the presence of small hemorrhages and petechiae. Arterial thrombi were present in the mucosa and submucosa of the stomach and intestines and appeared infrequently in the muscle layers.

The adrenal glands were studded with hemorrhages and the cortex was slightly atrophic. A few vessels were occluded by hyalin thrombi. The kidneys were studded with multiple hemorrhages. Thrombi were present in the medium-sized arteries. These lesions appeared to be organizing. (Fig. 2*b*.) The glomeruli showed an increase in the number of endothelial cells and the basement membrane and Bowman's capsule were thickened.

The grossly normal pancreas showed many thrombi in the arteries and arterioles accompanied by endothelial proliferation and early organization.

The lymph nodes contained many arteriolar thrombi. In one of the peribronchial lymph nodes there were many giant cells which resembled megakaryocytes.

The bone marrow was hyperplastic and the walls of a few vessels contained thrombi or hyalin bodies. (Fig. 2*c*.) Most of these appeared to be at a more advanced stage of development than those seen elsewhere. Megakaryocytes were numerous.

The brain exhibited a moderate degree of edema. Perivascular and perineural spaces were distended, with small hemorrhages about the smaller vessels. A few perivascular spaces contained phagocytes filled with iron pigment. No arterial thrombi were noted in the many sections examined.

Multiple thrombotic lesions with scattered hemorrhages were noted in sections from the psoas and rectus abdominis muscles.

The final pathologic diagnosis included the following: (1) malignant thymoma of the anterior mediastinum with invasion of the pericardium and metastasis to the mediastinal

lymph nodes and right lung; (2) thrombotic thrombocytopenic purpura with multiple thrombi in various organs; (3) multiple hemorrhages of all organs; (4) splenomegaly with multiple infarcts and (5) organized bronchopneumonia.

CASE II. A male railway employee, forty-eight years of age, was admitted to the clinic and was hospitalized twenty-four hours after an episode of confusion during which he had driven his car on the wrong side of a busy street. He complained of headache on the right side. His persistent confusion made an accurate history of his illness difficult to obtain. However, relatives stated that the patient had not felt well for a period of two months. For three weeks he had seemed pale and "yellowish," and had noted that he bruised easily. There was no known history of drug administration, allergy, or other significant disease involving the patient or members of his family.

On admission his temperature was 101°F. and blood pressure was 125 mm. of mercury systolic and 75 diastolic. The patient was disoriented and confused, and he appeared jaundiced. Numerous petechiae and ecchymoses were scattered over the trunk and extremities. There was a left hemiparesis with conjugate deviation of the eyes to the right. No significant abnormalities were seen in the ocular fundi. There was no palpable enlargement of liver, spleen or lymph nodes.

Laboratory examination revealed anemia, the value for hemoglobin being 6.6 gm. per 100 cc. of blood and for erythrocytes 2,170,000 per cu. mm. Values for leukocytes varied from 9,100 to 25,200 per cu. mm. of blood and a single determination of platelets gave a value of 30,000. Examination of stained smears of the blood disclosed the presence of occasional microspherocytes, extensive polychromasia and rather numerous normoblasts. There was a shift to the left of the myeloid elements which extended as far as the level of progranulocytes. Reticulocytes were present in large numbers (31.2 per cent). The bleeding time was prolonged to 17 minutes, but coagulation time was normal. Prothrombin times (Quick) ranged from 23 to 28 seconds.

A flocculation test for syphilis gave negative results as did Coombs' test. The titer of complement in the serum was 1:64 and heterophile antibody was present to a titer of 1:32. There were no cold agglutinins. The bromsulfalein test for liver function revealed retention of dye, grade 1. Other tests of liver function (thymol turbidity, zinc sulfate turbidity and cephalin-cholesterol flocculation) gave normal results.

Studies of chemical constituents of the blood gave the following results: 5.0 gm. of serum albumin per 100 cc. and 2.6 gm. of serum globulin. The value for urea ranged from 80 to 112 mg. per 100 cc. The concentration of potassium was 3.3 mEq./L.

Urinalysis revealed albuminuria, grade 2 (on the basis of grade 1 to 4 where grade 1 is minimal and grade 4 maximal), and microhematuria, grade 2.

Roentgenographic studies of the thorax and head gave normal results.

A provisional diagnosis of idiopathic acquired hemolytic anemia with associated thrombocytopenia was made. However, the prominent neurologic aspects of the case, attributed to hemorrhagic encephalopathy, suggested the possibility of thrombotic thrombocytopenic purpura.

Treatment with 200 mg. of cortisone and 600,000 units of penicillin daily was instituted. Four days later the platelets numbered 71,000 per cu. mm. of blood and the bleeding time was 6½ minutes. The patient's mental state, however, ranged from confusion to transient coma, and nystagmus of central type developed. Four transfusions of 500 cc. of blood failed to alter the hemoglobin or erythrocyte values.

On the ninth day after admission, following previous unsuccessful attempts because of the patient's inability to cooperate, a specimen of the sternal bone marrow was obtained by needle aspiration. The myeloid-erythroid ratio was 1:2.07. Study of films stained by Wright's method revealed a very cellular preparation with normoblastic erythropoiesis and myelopoiesis both of which were shifted to the left. Megakaryocytes appeared adequate in number, although shifted to the left, and showed little evidence of normal production of platelets. Reticuloendothelial phagocytosis of erythrocytes and pigment granules was noted. Paraffin sections made from solid marrow particles removed in the course of needle aspiration and stained with hematoxylin and eosin revealed hyperplastic marrow. The blood vessels were conspicuous because of moderate endothelial proliferation and dilated segments with finely granular thrombi occluding the lumina. (Fig. 3.) Fibroblastic proliferation was noted throughout the substance of some of the thrombi. Study by

FIG. 3. Case II. Section of biopsied bone marrow. Note saccular dilatation of blood vessel and finely granular thrombotic material invaded by fibroblasts. Endothelial proliferation not as marked as in Figure 1; hematoxylin and eosin × 365.

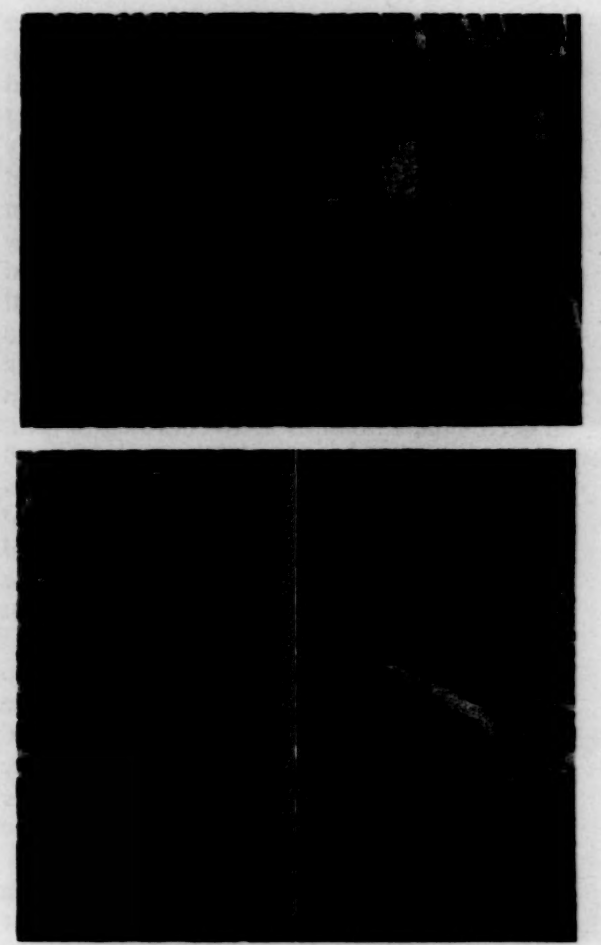

FIG. 4. Case II. (*a*) Heart; multiple thrombi in vessels with organization in one. Note hyalin body in wall of smaller vessel; hematoxylin and eosin × 250. (*b*) Adrenal; hyalin body in wall of vessel with elevation of endothelium; hematoxylin and eosin × 320. (*c*) Brain; multiple thrombi with focal areas of degeneration in perivascular spaces; hematoxylin and eosin × 300.

means of special stains failed to reveal the exact nature of the thrombotic material. These findings were regarded as corroborative evidence for the diagnosis of thrombotic thrombocytopenic purpura.

On the tenth day melena appeared. Thereafter the condition of the patient progressively deteriorated until he suddenly became comatose and died two weeks after admission.

Necropsy examination was performed two hours after death. Petechiae and ecchymoses were scattered throughout the skin. Petechiae were present in the peritoneum, pericardium, larynx, trachea, lungs, capsule of the liver, gastrointestinal tract and brain.

The heart weighed 410 gm. The pericardium was studded with petechiae and scattered ecchymoses. There was 75 per cent occlusion of the right coronary artery at a point 6 cm. from its origin which was the result of atherosclerosis, and which had caused the formation of numerous small healed infarcts. Almost all of the smaller arteries contained multiple hyalin-like bodies in the walls or extended hyalin masses with thrombi. (Fig. 4*a*.) Areas of degeneration of the myocardium were infrequent when compared to the large number of thrombi. Superimposed on many of the hyalin bodies in the walls of the vessels were recent thrombi which appeared to be propagating. Some of the occluding masses were undergoing organization or had been organized and recanalized. Study of sections stained by the Mallory phosphotungstic acid method revealed a few strands of fibrin in some of the thrombi. Platelets were infrequently seen.

The lungs exhibited numerous petechiae on the pleural surfaces. Focal regions of bronchopneumonia and hemorrhage were scattered throughout both lungs. The patchy bronchopneumonia was accompanied by purulent

bronchitis. The alveolar walls were infiltrated with lymphocytes and plasma cells. Many of the alveolar arteries were occluded. Some arterial walls showed hyalin masses under an intact endothelium. The hyalin mass occasionally protruded into the arterial lumen with elevation of the intact endothelium over the surface. In some instances the hyalin masses were completely extruded into the lumen of the vessel and were surrounded on all surfaces by endothelial cells. Many of these hyalin bodies appeared to be the site of origin of secondary thrombi made up of fibrin, fibroblasts and rarely platelets. In an occasional thrombus active fibroblastic proliferation could be seen. Others demonstrated complete organization with recanalization. Accompanying the thrombi and weakened arterial walls were saccular and fusiform aneurysmal dilatations. Occasional multinucleated giant cells resembling megakaryocytes were present in the capillaries of the alveolar walls.

The congested spleen weighed 195 gm. Throughout the sinuses and the pulp were numerous phagocytes containing erythrocytes. This erythrophagocytosis was accompanied by deposits of hemosiderin in the mononuclear phagocytes. A few arterial thrombi were seen.

The liver weighed 2,135 gm. Microscopic study revealed increased granularity of the hepatic cells with condensation of pigment in the cells near the central vein. Many of these cells were necrotic. Reticuloendothelial cells were numerous and contained iron pigment. In the periportal spaces many vessels were involved by the thrombotic process, which was in various stages of development.

Numerous petechiae were present in the esophagus and stomach. The latter contained partially digested blood. An acute ulcer of the duodenum measured 0.5 cm. in diameter. Arteriolar thrombi were scattered throughout the mucosa and there was infarction of the superficial layers.

The pancreas was normal on gross examination. Hyalinized vessel walls and occluding thrombi were scattered throughout the substance of the pancreas with very little evidence of hemorrhage.

The lymph nodes showed reticuloendothelial hyperplasia associated with erythrophagocytosis, and numerous phagocytes contained hyalin and fat droplets. The arteries of the lymph nodes had thrombi and hyalin masses in the walls. The thyroid and parathyroid glands showed arteriolar thrombi throughout their structures.

The cortex of the adrenal gland showed depletion of lipoid material and atrophy. Hemorrhages were scattered throughout the cortex and medulla. Many arteriolar thrombi in all stages of development and organization were present. (Fig. 4*b*.)

The kidneys were slightly enlarged. The glomeruli exhibited proliferation of endothelial cells with thickening of Bowman's capsule. The afferent arterioles were hyalinized, thickened and revealed changes similar to those seen in amyloid disease. Study of sections of these vessels with special stains revealed no amyloid. Many of the granular tubules were undergoing necrosis. Granular, hyaline and pigment casts were seen in the tubules.

The bone marrow was hyperplastic. Numerous phagocytes containing iron pigment were scattered throughout the marrow. The smaller vessels were occluded by hyalin masses or thrombi or both. Megakaryocytes were normal in number. Erythrophagocytosis was present.

The brain was edematous. The cut surfaces revealed multiple petechiae and depressed perivascular areas. Many of the vessels in the brain were occluded by thrombi, most of which were associated with hyalin masses within the vessel walls. (Fig. 4*c*.) The occlusions of the vessels resulted in small infarcts. There were many more infarcts in the brain than in other organs. The pituitary gland showed several infarcts accompanying occluded vessels.

Thrombotic lesions and scattered hemorrhages were observed in sections from the psoas and rectus abdominis muscles. The sections of skin showed no thrombotic lesions.

The final pathologic diagnosis included the following: (1) thrombotic thrombocytopenic purpura with multiple thrombi in the heart, lungs and other organs; (2) petechiae and hemorrhages in the lungs, brain and other organs; (3) multiple small infarcts of the heart and brain and (4) bilateral bronchopneumonia.

COMMENTS

Clinical Features. While the over-all clinical features of both cases seemed to justify the presumptive diagnosis of thrombotic thrombocytopenic purpura, some aspects of the first case were atypical. The true nature of the pulmonary lesion in this patient was not established during life. Whether the locally invasive

thymoma, which presented widespread areas of necrosis, was causally related to the development of the presenting syndrome is a matter of speculation. Instances of antecedent or coexisting syndromes of "arthritis"[7] clinically resembling disseminated lupus erythematosus,[20] tuberculosis and proliferative glomerulitis,[13,22] have been recorded but we are not aware of previous reports of the occurrence of this syndrome in association with neoplastic disease. It is conceivable that the disorder in this patient was initiated in response to the products of tumor necrosis.

While stupor and disorientation succeeded by coma and generalized convulsions were prominent manifestations in the first case, careful study of multiple sections of brain tissue revealed none of the characteristic vascular lesions. The pathologic picture was rather that of cerebral edema with scattered small hemorrhages compatible, in a non-specific manner, with the findings which might be anticipated in a febrile illness complicated by severe anemia and thrombocytopenia.

Laboratory Features. Fortuitously perhaps, needle aspiration of the sternal bone marrow done only once in each case provided bits of marrow tissue in sections of which the characteristic lesions of thrombotic thrombocytopenic purpura were readily demonstrable. It may be reasonably expected that multiple aspirations may be necessary to accomplish this when lesions are not widely disseminated throughout the marrow substance. Preparation of ordinary films of the marrow cannot be expected to yield specific diagnostic information of this type.

Muscle biopsy may be helpful in some instances. Characteristic lesions were present in all sections of skeletal muscle examined following necropsy in our cases.

Early observers attributed the characteristic thrombocytopenia of this syndrome to the peripheral dissipation of platelets in the formation of multiple "platelet" thrombi. Meacham and co-workers,[21] however, have emphasized the inconclusive nature of the evidence cited in support of this concept. Our studies, in agreement with observations of Gore[18] and Orbison,[23] indicate that the occluding thrombi are most often derived from subendothelial deposits of hyalin-like material. Thrombi containing platelets and fibrin may develop at such sites of endothelial involvement.

In stained films of blood obtained from the marrow megakaryocytes appeared in normal or increased numbers. The majority of these cells appeared immature and overt evidence of normal formation of platelets was absent. We are inclined to share the opinion that the thrombocytopenia in these patients is chiefly the result of underproduction rather than overutilization of platelets. However, the possibility of a combination of these mechanisms cannot be denied.

All available evidence seems to support the view that the anemia in these patients is hemolytic in origin. However, neither our observations nor those recorded in the literature elucidate the precise mechanism involved. Increases in fragility of erythrocytes have been inconsistently demonstrated and efforts to demonstrate abnormal plasma factors have been generally fruitless. The positive results of the serologic tests for syphilis encountered in our first patient were apparently caused by biologic false positive reactions. This phenomenon might suggest an associated immunologic disturbance but these tests provide no information of specific value. Similar serologic reactions were noted in the cases of thrombotic thrombocytopenic purpura reported by Brown and Norman.[7]

Reticuloendothelial hyperplasia and erythrophagocytosis were prominent features noted on histologic examination of the hematopoietic tissues, suggesting, as does the absence of hemoglobinuria in reported cases, that the site of blood destruction is not intravascular.

Necropsy revealed that, aside from the evidence of reticuloendothelial hyperplasia and erythrophagocytosis just mentioned, the most significant changes were those characteristically involving the vessels. In both cases a peculiar, focal, hyalin-like change in the walls of the small arteries and arterioles appeared to be the initial lesion. In association, partial or complete occlusion of the involved vessel occurred either by (1) encroachment on the lumen by protrusion of the mural lesion, the endothelial lining of the vessel remaining intact; or (2) extrusion of the hyalin-like substance from the wall into the lumen; or (3) propagation of a fibrin or platelet thrombus from the site of endothelial damage or rupture associated with a mural lesion or (4) propagation of thrombus on an extruded mural mass. The occluding masses exhibited varying degrees of organization ranging from infiltration by a few proliferating fibroblasts to complete obliteration of the

lumen with recanalization of an organized thrombus. The lumina of the vessels were incompletely occluded in most instances. As a result associated infarction was infrequently observed except in the brain and myocardium, tissues which are sensitive to impairment of their blood supply. The kidneys, spleen, lymph nodes and lungs presented the greatest number of thrombotic vessels but no organs were free of involvement.

The material in the walls of involved vessels is not unlike the hyalin material seen in the kidneys in disseminated lupus erythematosus and it resembles the hyalin bodies described by Klemperer. There was no evidence of arteritis with the exception of the rare occurrence of secondary changes in areas of infarction. Many of the vessels which were involved presented saccular and fusiform aneurysmal dilatations of the type described by Orbison.[23]

Whether the vascular lesions are primary or are secondary to the effects of intraluminal formation of thrombi from platelets has been a subject of controversy. Our observations lead us to hold the former view. The concept that the thrombotic lesions in this disease are composed of platelets or platelet material is based largely on studies of the reaction of sections of the involved tissues to various stains. Orbison[23] pointed out that no known stains have a specific or exclusive affinity for platelets and that the strictly intramural lesions react in exactly the same way as do the platelets and intramural occlusive material when these lesions are exposed to the same stains.

Occasional petechial hemorrhages in areas independent of vascular occlusion were observed and were attributed to the associated thrombocytopenia and possibly also to the morphologically insignificant effect of the unidentified vascular "toxin" responsible for the characteristic lesions.

Pathogenesis. The cause of this spectacular disturbance remains undetermined. The possibility of an underlying reaction of hypersensitivity, particularly to drugs, has been suggested in some reports. At present, evidence in support of such a concept seems fragmentary. In some instances the syndrome has appeared to follow infections, particularly of the respiratory tract, but there is no evidence that would implicate a specific bacterium or virus as a direct cause of thrombotic thrombocytopenic purpura.

There is no evidence to suggest a cause and effect relationship between the hematologic and the vascular aspects of this syndrome. Quite likely they represent independent responses to the causative mechanism.

Pathologic and clinical similarities between this process and disseminated lupus erythematosus, which is occasionally complicated by the development of hemolytic anemia and thrombocytopenia, might be pointed out but the differences are equally striking. Of some interest was the absence of "L. E. cells" in the preparations of marrow that were examined in our cases. Nevertheless, the syndrome appears to be most readily catalogued at present as a new member of the group of so-called collagen diseases.

Perhaps establishment of the diagnosis at an earlier stage of the process, as seems possible by study of sections of the marrow as described herein, may allow more detailed consideration of the natural history of the disease and give rise to appropriate clinical investigation of its pathogenesis.

Treatment. All cases thus far reported have terminated fatally. Splenectomy as a last resort has been uniformly futile. Interestingly, splenectomy in the case reported by Meacham and associates[21] resulted in improvement and survival for three years. At the time of splenectomy this patient presented the relatively common problem of idiopathic acquired hemolytic anemia without the other clinical features usually associated with thrombotic thrombocytopenic purpura. It seems unlikely, however, that splenectomy can be expected to offer more than temporary palliation at best.

The results of treatment with corticotropin or cortisone in our cases and in the case reported by Meacham and associates[21] were unimpressive. However, administration of these agents at an earlier stage of the disease or for more extended periods should be considered.

The course of the disease has not been measurably influenced by employment of various supportive measures including multiple blood transfusions.

SUMMARY

Two cases of thrombotic thrombocytopenic purpura are reported in which the clinical diagnosis was verified before death by demonstration of typical vascular lesions in microscopic sections of aspirated bits of sternal bone marrow.

Our experience demonstrates a readily available and relatively simple means of establishing a clinical diagnosis which heretofore has been made only with difficulty.

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Rupture of Echinococcus Cysts into the Bile Ducts Simulating Stones in the Common Duct*

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THE rupture of a hydatid cyst of the liver into the biliary passages, producing a symptom complex characterized by anaphylaxis, urticaria followed by severe epigastric colic, chills, fever and jaundice simulating obstructive cholelithiasis, is well known in the countries where echinococcosis is prevalent. Perusal of the standard textbooks of tropical medicine reveals no description of this syndrome. Belding[1] mentions the urticarial reactions associated with rupture of the cyst but fails to describe the other components of the syndrome. Faust,[2] Manson,[3] Stitt[4] and Culbertson[5] make no reference to this clinical pattern. Bockus[6] mentions the rupture of a cyst into the biliary ducts producing pain, jaundice and a "virulent" cholangitis. The best and most complete description of this entity is found in the comprehensive monograph by Dew.[7] Only a few papers dealing with this subject could be found in the North American literature,[8–12] and these were derived principally from the experiences of foreign authors.[13,14]

With recent shifts in world population and the arrival of immigrants from areas where hydatid disease is prevalent it is expected that the disease will be more frequent in this continent.

It is our purpose to direct attention to this syndrome and to report our experience with two cases in immigrants. Since this syndrome simulates biliary colic due to stones in the common duct, an awareness of the symptom complex will enable the physician to establish the diagnosis and institute the proper surgical approach.

Poore et al.[15] reviewed the case records of all patients with echinococcus cysts seen at the Mayo Clinic up to 1949. The liver was the primary site in forty patients. The diagnosis in this group was based on history, skin test, clinical findings, roentgenograms of the liver, or on the finding of hydatid cysts at surgery. Fourteen (35 per cent) of the patients gave a history of jaundice during some of their attacks of biliary pain. Ten (25 per cent) of the forty gave a history of vague right upper quadrant pain. Poore[15] reported an additional typical case in an itinerant fifty-four year old Greek immigrant who had symptoms of recurring jaundice, chills and fever with severe epigastric pain. The eosinophil count was 34 per cent. The skin and complement fixation tests were positive. At surgery, a large echinococcus cyst of the right lobe of the liver was found, with three daughter cysts up to 1.5 cm. in diameter obstructing the common duct. The patient made an uneventful recovery. Poore[15] reported that less than 25 per cent of the cases he reviewed had a significantly elevated eosinophil count. Dew[7] discusses the various types of severe anaphylaxis due to sudden release of large amounts of protein-containing fluid from the cysts. He emphasizes that if an accurate history of all cases of hydatid cysts containing daughter cysts were obtainable, it would be found that the patients had suffered at one time or another from obscure attacks of pain, frequently associated with urticarial eruptions. He believes that the colicky nature of the pain of rupture is due either to a suppurative process with or without hepatitis or to the passage of débris or membranes down the bile ducts. He points out that in some cases the sudden onset of pain caused by rupture into a biliary duct is the first indication of the disease. He believes jaundice to be present in 80 per cent of the patients and makes the startling statement that in Australia "the occurrence of jaundice with hepatomegaly is more likely to be due to hydatid disease than to migrating stones."

Oosthuizen[14] states that in the liver a majority of cysts are silent but that they may leak into the bile duct, discharging their contents and leading to an obstructive jaundice with biliary colic which is usually mistaken for calculus cholecystitis. Perkins[10] mentions in a case report that rupture may occur with chills, fever and

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jaundice. Gennaro[8] reported a case in a thirty-four year old Italian female and cautions that the frequent errors connected with the diagnosis are due to the lack of awareness of the existence of the disease in North America. He describes the typical symptomatology of the syndrome in detail. The case of a twenty-five year old Greek housewife with symptoms of jaundice, epigastric pain, nausea, vomiting and fever is recorded in the Case Records of the Massachusetts General Hospital.[11] Arce[13] states that rupture of the cyst into the biliary system may give rise to biliary colic or to obstruction of the bile duct by daughter cysts, fragments of membranes, etc. Weinberg[12] reported the case of an American-born white male who apparently contracted the infection in France during World War I. Typical symptoms of intermittent common duct obstruction were present.

CASE REPORTS

CASE I. This forty year old white male physician who had emigrated from Palestine was admitted to Mount Sinai Hospital on December 18, 1939,* complaining of attacks of severe epigastric pain with vomiting, of three weeks' duration. He gave a history of vague abdominal discomfort following meals for the past two years.

On the evening following admission he suffered chills and fever for several hours and his urine became dark. His temperature was 100°F., blood pressure 142/90, pulse 74, respirations 20. The skin and conjunctivae were moderately icteric. The liver, kidney and spleen were not palpable. There was moderate tenderness in the epigastrium. The icteric index was 35. A diagnosis of acute cholecystitis with obstructive jaundice and cholangitis due to calculus in the common duct was made. His symptoms and jaundice quickly subsided and he was discharged on December 26, 1939, with no complaints. His second admission was January 11, 1940, at which time he complained of severe right upper quadrant colicky pain of three days' duration. He appeared moderately ill with a temperature of 103°F., pulse 104, respirations 26, blood pressure 130/72. There was considerable tenderness in the epigastrium. He again appeared visibly jaundiced. On the following day he had a violent chill and the jaundice deepened. Again a diagnosis of the "Charcot syndrome" of intermittent cholangitis with stones in the common duct was made. The jaundice gradually decreased and the liver became palpable 5 cm. below the costal margin. During the course of his illness a persistent eosinophilia up to 35 per cent was observed. On January 26th laparotomy was performed through a right upper quadrant rectus incision. A large echinococcus cyst of the left lobe of the liver was revealed and several small daughter cysts were found upon exploration of the common duct. No cysts were found within the gallbladder although the mucosa was edematous. Wet smears from the specimens obtained showed typical echinococcosus scolices with hooklets. The patient made an uneventful recovery and was discharged on February 21, 1940.

CASE II. This forty-two year old white male Hungarian immigrant janitor was admitted to the Mount Sinai Hospital* on November 6, 1950, because of sudden onset of severe epigastric colicky pain with vomiting. He was treated for severe generalized urticaria one week prior to admission, with complete alleviation of symptoms following treatment with antihistaminic drugs. The patient was visibly icteric and there was considerable tenderness in the right upper quadrant. The liver edge was palpable two to three fingers below the right costal margin and was smooth, firm and moderately tender. A diagnosis of common duct obstruction due to stone was made. Acute pancreatitis was also considered as a possibility. Epigastric pain and vomiting were continuous in the first few days of the hospital course.

The white blood count on November 12th was 34,000 with 75 per cent eosinophils, and a diagnosis of echinococcus cyst of the liver with rupture into the common duct was suspected. The cephalin flocculation test was persistently 4+ positive and the thymol turbidity was 3 units; 4.1 mg. urobilinogen were excreted in the urine during a twenty-four-hour period on November 14th and 4.9 mg. on December 1st. The corrected Westergren sedimentation rate was 28 mm. in one hour. Stool analysis revealed large numbers of eosinophils but no parasites or cystic forms. Graham-Cole examination failed to visualize the gallbladder, and no calculi could be seen on a flat plate of the abdomen. On November 17th the white count fell to 19,400 with 75 per cent eosinophils. The RBC was 4,990,000 and the platelets 207,000. The hemoglobin was 15.6 gm. per 100 per cent.

* Service of Drs. I. M. Trace and A. Strauss.

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The highest blood cholesterol value was 264 mg. per cent with 169 mg. per cent esters. The maximum total serum bilirubin content was 3.6 mg. per cent. The icteric index, which reached 38, subsided to normal range before the patient was discharged on December 9th. Sternal marrow aspirated on November 17th was considered normal except that the majority of the granulocytic cells were eosinophils and constituted over 70 per cent of the nucleated forms. On November 25th the patient was still jaundiced and the liver size was unchanged. Complement fixation test for echinococcosis was positive. The Casoni skin test with fresh antigen of echinococcus gave a wheal 1.5 cm. in diameter, with a 3 cm. erythema zone, and was interpreted as positive. Surgery was refused by the patient. He was discharged on December 9, 1950.

The patient was admitted to another hospital on May 16, 1951, complaining of severe right upper quadrant cramping pain radiating to the back with constant vomiting of two days' duration. A small mass was palpable in the right upper quadrant and a diagnosis of empyema of the gallbladder was considered. Surgical exploration of the abdomen revealed a large hydatid cyst of the right lobe of the liver, the size of an orange, and acute cholecystitis with daughter cysts in the common duct and gallbladder. Cholecystectomy was performed, the hydatid cyst was evacuated and the cavity packed with iodoform gauze. The patient made an uneventful postoperative recovery except for a temperature rise to 102°F. on the third postoperative day. He was discharged ten days after admission. Subsequent examination of the patient revealed him to be in good health, with no abnormal findings. The red blood count was 4,790,000, hemoglobin 14.5 gm. per cent, white blood count 8,450,000. The differential count was normal and there was no eosinophilia.

COMMENT

Only two points serve to differentiate this syndrome of "pseudocholelithiasis" from true calculi in the biliary ducts: (1) anaphylactic or urticarial reactions and (2) eosinophilia. Although present in both of our cases, eosinophilia according to Dew[7] is found in only 25 per cent of the patients with this disease. It has been categorically stated that echinococcal disease is always contracted outside of the continental limits of the United States. All cases in the literature confirm this axiom. The disease may be overlooked because of the long latent period between the time of contraction of the disease and its clinical appearance.

SUMMARY

Attention is directed to the syndrome of "pseudocholelithiasis" due to the rupture of echinococcus cysts into the bile ducts. The salient features of this symptom complex are anaphylactic or urticarial symptoms, intermittent chills, fever and obstructive jaundice. Marked eosinophilia may occur. Two cases of the syndrome are presented.

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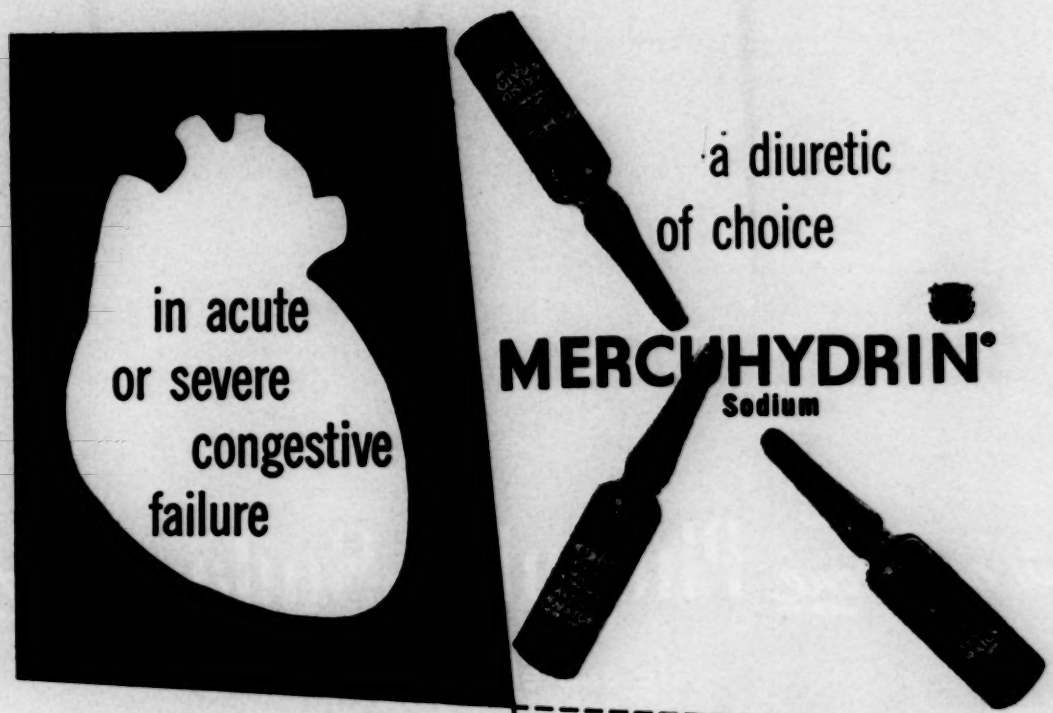

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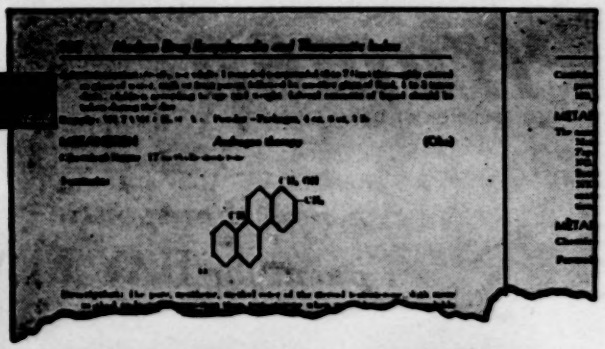

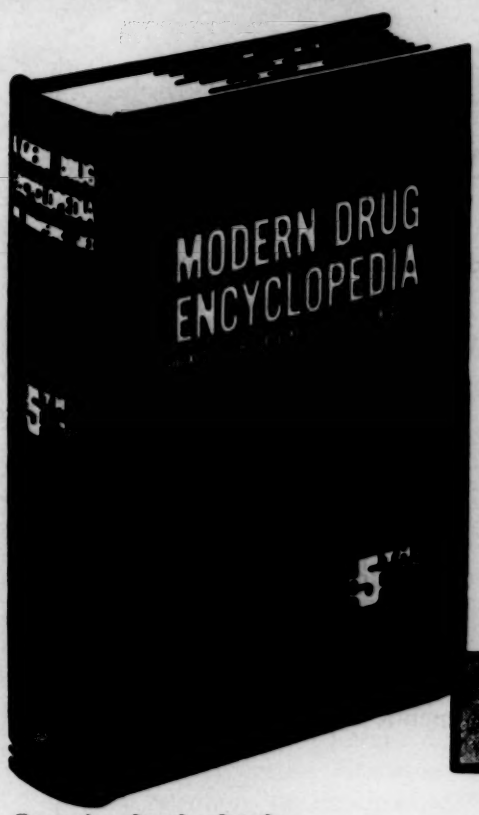

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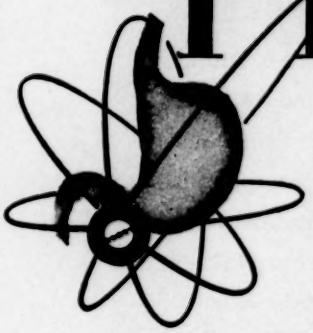

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PRANTAL* Methylsulfate is a member of an entirely new class of synthetic anticholinergic compounds. It curbs excessive vagal stimuli to the stomach by inhibiting synaptic transmission across parasympathetic ganglia.

PRANTAL Methylsulfate is unique among anticholinergic compounds. Because of its selective action, doses which reduce gastric motility and secretion rarely cause dilatation of the pupils, dryness of the mouth, urinary retention, or constipation.

The pharmacodynamics of PRANTAL Methylsulfate have been the subject of extensive laboratory investigations in which the classical procedures were used. Studies by leading clinical investigators have confirmed the value of its unusual properties in treatment of the peptic ulcer syndrome.

A Clinical Research Division monograph is now in press and will be sent to you promptly on request.

A clinical supply of PRANTAL Methylsulfate will be sent to you on request.

Average Dosage: One tablet (100 mg.) four times daily.

Packaging: PRANTAL Methylsulfate (brand of diphenmethanil methylsulfate), 100 mg. scored tablets, bottles of 100.

*T.M.

Schering CORPORATION • BLOOMFIELD, NEW JERSEY

PRANTAL

for Control of Hypertension

Apresoline™

Hydrochloride
(brand of hydralazine hydrochloride)

Apresoline is a relatively safe, *single* antihypertensive drug with no serious untoward reactions, providing benefits in many cases — complete control in some. It is recommended that Apresoline be used in those hypertensive patients who have not been adequately controlled by conventional regimens (diet, mild sedation, rest, etc.). The following important considerations should be of interest in general practice:

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Provides gradual and sustained reduction of blood pressure with no dangerous, abrupt fall on oral administration.

Affords uniform rate of absorption and infrequent dosage adjustments.

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Side effects often disappear as therapy is continued or can be ameliorated with adjunctive medication.

Produces significant relaxation of cerebral vascular tone.

Complete information regarding manner of use and clinical application available on request.

Ciba

Ciba Pharmaceutical Products, Inc., Summit, New Jersey

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When taken about half an hour before meals, orange or grapefruit juice is highly effective in helping overweight patients to adhere to their reducing regimens. Citrus has "very definite advantages"* as an appetite appeaser. It helps to reduce the demand for high caloric foods, and supplies readily utilizable carbohydrates to combat hypoglycemia. It is economically available in homes or restaurants. And, of no small consideration, most everyone likes orange or grapefruit juice.

**Postgrad. Med. 9:106, 1951.*

FLORIDA CITRUS COMMISSION • LAKELAND, FLORIDA

FLORIDA *Citrus*

ORANGES • GRAPEFRUIT • TANGERINES

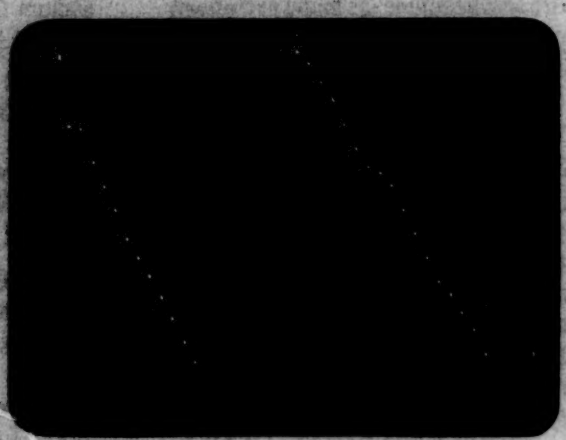
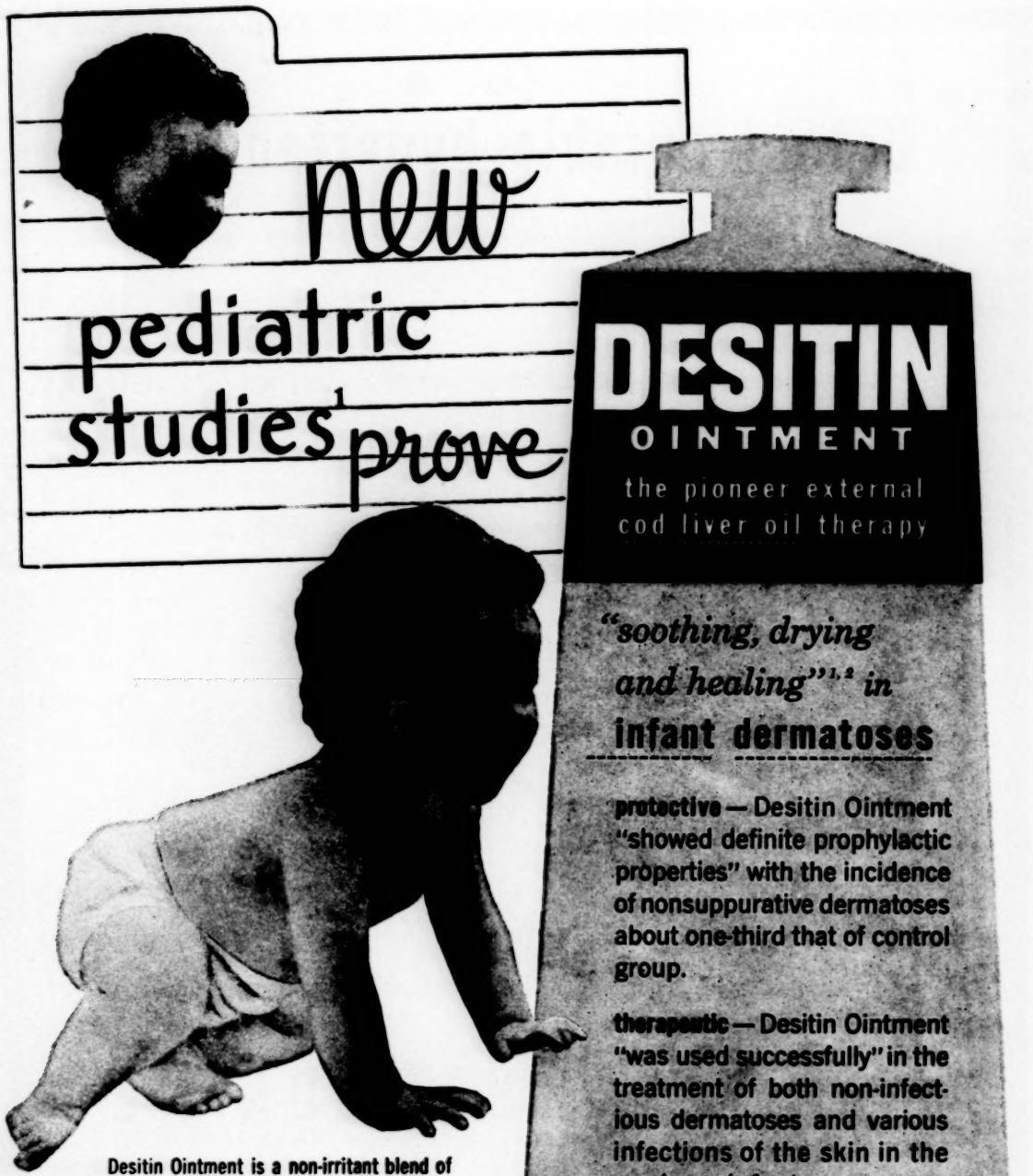


CHART OF WEIGHT LOSS
BROKEN LINE—OBSERVED LOSS • SOLID LINE—PREDICTED LOSS



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70 Ship Street • Providence 2, R. I.

1. [illegible], C. B., Grayzel, H. G., and Kramer, B.: Archives of Pediat. 68:382, 1951.
2. [illegible], H. T., Cowles, F. C., Bobroff, A. and Leviticus, R.: Ind. Med. & Surg. 18:512, 1949.

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New **Steroid-Vitamin-Mineral**

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Your patients — young or aged — who need steroidal-nutritional support will have it in a single tablet when you prescribe Covisten. For the young, Covisten will prove invaluable as an anabolic tool — for the aged, an excellent way to supplement waning gonadal function and diminishing intake or utilization of foodstuffs. Covisten supplies Stenediol® (methyl androstenediol — the non-virilizing androgen which imparts the 'lift' unique with steroids) plus vitamins plus minerals in balanced combination.

Each tablet supplies:

Stenediol (methyl androstenediol)	5 mg.
Ascorbic acid	50 mg.
Thiamine HCl	2 mg.
Riboflavin	3 mg.
Pyridoxine HCl	1 mg.
Vitamin B_{12}	2.5 mcg.
Niacinamide	20 mg.
Folic Acid	0.5 mg.
Vitamin A	5000 U.S.P. Units
Vitamin D_3	500 U.S.P. Units
Ferrous Gluconate	75 mg.
Dibasic Calcium Phosphate	200 mg.
Manganese Hypophosphite	0.5 mg.
Dibasic Magnesium Phosphate	20 mg.

Bottles of 100 & 1000

Organon INC. • ORANGE, N. J.

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Complete relief of symptoms was obtained by most menopausal patients, Perloff reported, and the greatest percentage of patients who "expressed clearcut preferences for any drug designated 'Premarin.'"

Perloff, W. H.: Am. J. Obst. & Gynec. **58**:684 (Oct.) 1949.

"PREMARIN"® in the menopause

Estrogenic Substances (water-soluble) also known as Conjugated Estrogens (equine). Tablets and liquid.

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"Adoption of these control measures now is warranted by the evidence so far obtained" . . .

GERICAPS

(SHERMAN)

Each capsule supplies the true lipotropics (choline and inositol) approximately equivalent to one gram—choline dihydrogen citrate, also Vitamin A and the B-Complex factors, together with rutin and Vitamin C in adequate amounts.

● **The Gericaps formula makes possible a double use (Prophylactic and therapeutic) in the management of conditions of impaired metabolism of fat and cholesterol.**

The lipotropics in Gericaps enter into the bio-synthesis of phospholipids, helping to bring about a better *balanced ratio* of cholesterol and phospholipids, which has been suggested as more important than the actual cholesterol level itself.

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Gericaps contain therapeutic amounts of the factors to combat capillary weakness (rutin and Vitamin C) so often associated with abnormal cholesterol and fat metabolism.

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The *weight reduction problem*

a more complete solution . . .

OBEDRIN plus THE 60-10-70 DIET

Obedrin Tablets permit full utilization of the appetite depressing action of methamphetamine hydrochloride but eliminate the central nervous stimulation, so the patient does not suffer from nervous irritability and insomnia.

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Obedrin Tablets and the 60-10-70 basic diet will permit loss of weight with minimum discomfort, thus inviting patient cooperation.

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*Semoxydrine Hydrochloride	5 mg.
Pentobarbital Sodium	20 mg.
Ascorbic Acid	100 mg.
Thiamine Hydrochloride	0.5 mg.
Riboflavin	1 mg.
Niacinamide	5 mg.

*Methamphetamine Hydrochloride

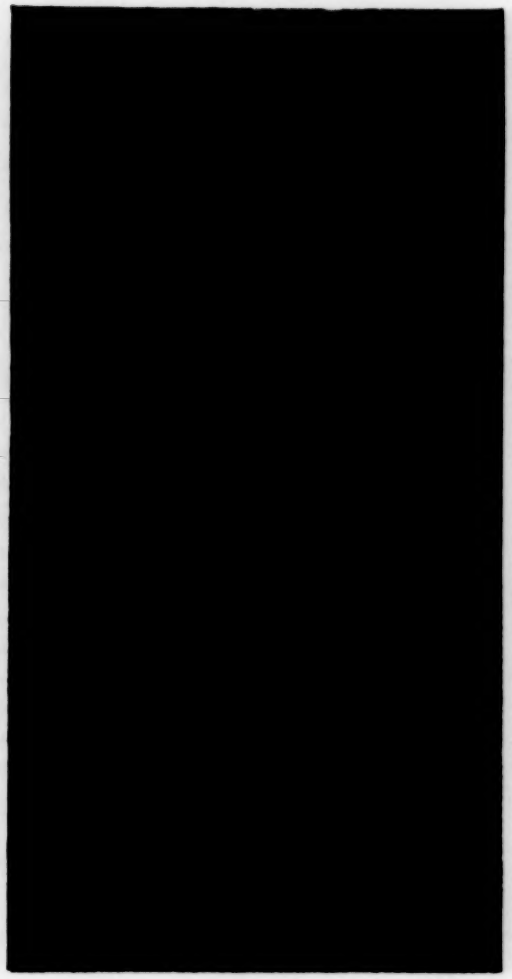
Obedrin is supplied in bottles of 100, 500 and 1,000 yellow grooved tablets.

A COMPLIMENTARY PAD OF DIET SHEETS AND A TRIAL SUPPLY OF OBEDRIN SENT TO PHYSICIANS ON REQUEST.

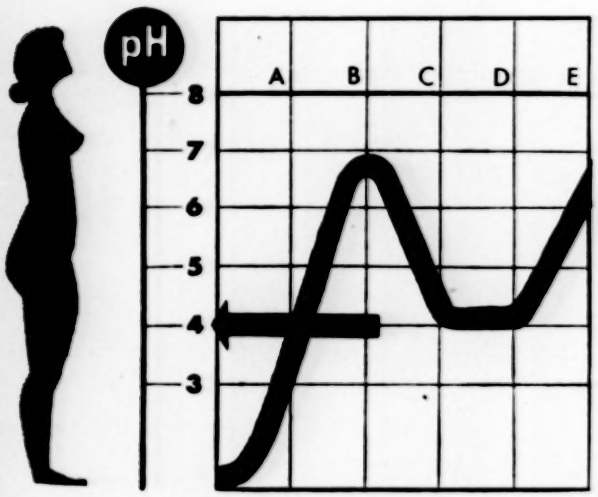
Obedrin

MASSENGILL BRISTOL, TENNESSEE

In Trichomonas infection . .



treatment "must not only include a trichomonacide, but it must furnish sugars to be stored as glycogen in the vaginal epithelium and provide a favorable medium for regeneration of the Döderlein's bacilli . . .".*



The normal adult vaginal mucosa is relatively thick, rich in glycogen and its secretions have an acidity within the range of pH 3.8 to 4.4. Glycogen is metabolized to lactic acid by the Döderlein bacilli, thus maintaining the normal acid state.

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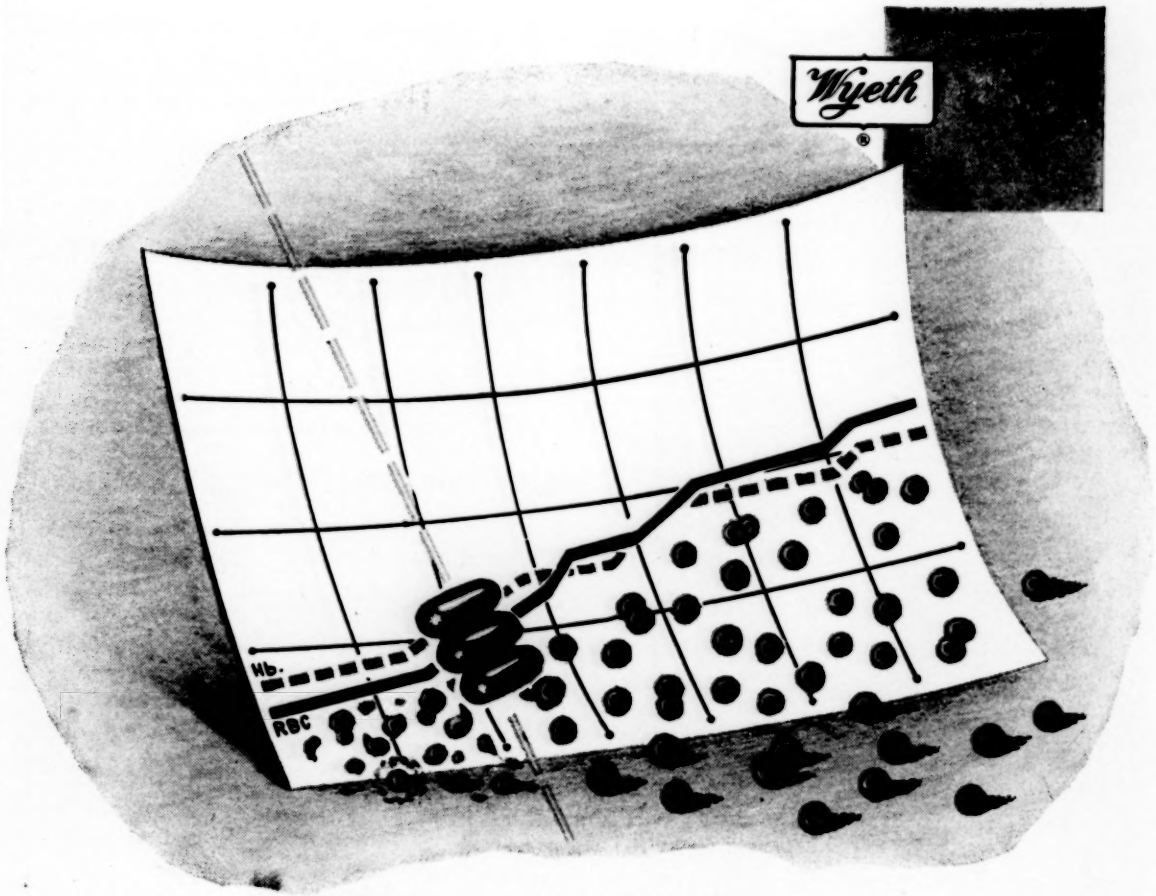
"We prescribe Floraquin tablets which contain Diodoquin . . . boric acid, and lactose and dextrose."*

Searle RESEARCH IN THE SERVICE OF MEDICINE

*Boehme, E. J.: Trichomonas Vaginalis Vaginitis; Diagnosis, Treatment, Causes of Failure in Treatment, S. Clin. North America 25:545 (June) 1945.



for all nutritional anemias . . .

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	Per daily dose (3 capsules)
Ferrous Sulfate, Dried	681 mg.*
Copper (as copper sulfate)	3 mg.
Vitamin B_1 (thiamine hydrochloride)	15 mg.
Vitamin B_2 (riboflavin)	6 mg.
Vitamin B_6 (pyridoxine hydrochloride)	3 mg.
Vitamin B_{12} (crystalline)	15 mcg.
Vitamin C (ascorbic acid)	225 mg.
Folic Acid	1 mg.
Calcium Pantothenate	3 mg.
Niacinamide	60 mg.
Liver, desiccated, N.F.	525 mg.

*Approximately equivalent to 15 gr. ferrous sulfate U.S.P. or 204 mg. of elemental iron

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IRON • VITAMINS • LIVER

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